EFFECT OF CITY WASTEWATER ON MUSTARD
(*Brassica juncea* L. *Czern & Coss.*)

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

BOTANY

DILSHADA TABASSUM

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2006

EFFECT OF CITY WASTEWATER ON MUSTARD (*BRASSICA JUNCEA* L. CZERN & COSS.)

by

DILSHADA TABASSUM

ABSTRACT

Abstract of the thesis submitted to the Aligarh Muslim University, Aligarh, India for the Degree of Doctor of Philosophy in Botany.

Four pot experiments were conducted on *Brassica juncea* var. Varuna during the rabi seasons of 2002-2005 at the net house of the Department of Botany, Aligarh Muslim University, Aligarh, India. All the experiments were carried out according to randomized complete block design. The data were mostly found significant and are given below.

Experiments I (2002-2003) was conducted to study the comparative effect of two concentrations of city wastewater, i.e. 50%WW and 100%WW and ground water (control) on mustard with four levels of nitrogen, i.e. N_0, N_{40}, N_{80} and N_{120}. 100%WW proved beneficial for most parameters including seed and oil yield. Among different doses N_{80} proved optimum as N_{120} was at luxury consumption and N_{40} was deficient. Among interactions 100%×N_{80} was best combination while 100%×N_{40} was equally effective as GW×N_{80} proving the utility of wastewater as a source of nutrients specially the nitrogen.

Experiment II (2002-2003) was conducted simultaneously with Experiment I to study the effect of wastewater treatments as in Experiment I in presence of four levels of phosphorus i.e. P_0, P_{15}, P_{30} and P_{45}. Application of P_{30} proved optimum, P_{45} luxury and P_{15} deficient for most of the growth, quality and yield parameters studied in earlier experiment. Among wastewaters 100%WW proved good. When interactions were considered 100%×P_{30} was the best while 100%×P_{15} was equally effective as GW×P_{30}.

Experiment III (2003-2004). In this experiment the performance of the same variety of mustard was studied under the same levels of water in presence of four levels of potassium, i.e. K_0, K_{10}, K_{20} and K_{30}. Again 100%WW proved best followed by 50%WW and GW. Better results were given by K_{20} whereas K_{10} proved deficient and K_{30} showed the luxury consumption. When the interactions were considered 100%×K_{20} proved good and this combination gave value at par to 100%×K_{30}, while 100%×K_{10} was at par with GW×K_{20} for various parameters including seed yield.

In Experiment IV (2004-2005) optimum doses obtained in I-III ($N_{80}P_{30}K_{20}$) and their half ($N_{40}P_{15}K_{10}$) in addition to $N_0P_0K_0$ were applied to the crop under same levels of irrigation as given earlier. In this experiment also 100% wastewater was more effective which was followed by 50%WW and GW. Optimum dose of NPK surpassed the half dose. When interaction was considered 100%×$N_{80}P_{30}K_{20}$ was the most effective combination. In this study also 100%×$N_{40}P_{15}K_{10}$ was at par with GW×$N_{80}P_{30}K_{20}$ confirming the beneficial use of wastewater for this crop and showing that the inorganic fertilizer can be saved.

It may be noted that noted that the physico-chemical characteristics excluding BoD and CoD were within permissible limits and also the heavy metals except Ni. However the microbiological tests of wastewater revealed that it contained some pathogenic bacteria since the crop is a seed crop and is not eaten raw, however the presence of these bacteria may be cause of concern for the farmer's so requires some precautions.

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"Plants speak to men but only in whisper; their voice can be heard only by those who remain close to them".

N.E. Borlaug

Dedicated to My Dear Parents and Brothers

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CERTIFICATE

This is to certify that the thesis entitled, "Effect of City Wastewater on mustard (*Brassica juncea* L. Czern & Coss.)" submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in **Botany** is a faithful record of the bonafide research work carried out at the **Aligarh Muslim University, Aligarh** by **Miss Dilshada Tabassum** under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.

(ARIF INAM)
Supervisor of research

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I am sincerely thankful to Mr. Rais Ahmad Khan for typing this thesis without any delay.

With all the faith in Almighty I place this work in the hands of my examiner with the hope that he will bear with the shortcomings that might have crept into this thesis inadvertently.

(Dilshada Tabassum)

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INTRODUCTION

Chapter 1

INTRODUCTION

The productivity of a crop depends upon several factors of which the application of inorganic fertilizers and irrigation are at the top in addition to the type of crop and its cultivar. However, the use of fertilizers beyond limits in addition to being uneconomical is potentially harmful to the environment as these get leached through the soil beyond the rootzone eventually reaching the groundwater or escape because of the surface runoff into water bodies and cause pollution over a period of time. Therefore, preserving quality and availability of fresh water resources are the most pressing among many environmental challenges of today perhaps because water is considered as a "life support system" and people fail to realize the intensity of its degradation. The fast growth of human population and urbanization and the increased industrial activity have lead to overloading of the carrying capacity of water bodies to assimilate and decompose wastes which also gave rise to deterioration of the water quality. As per the last estimate of CPCB, 423 class I cities and 498 class II towns of the country generated about 26250 million litres per day (mld) of wastewater while the wastewater generated from all major industries was 83048 mld (Trivedi, personal communication). This situation warrants the immediate redressal through the water resource management, therefore, there is need to bring the perceivable shift in our approach towards the water use.

The "Green Revolution" no doubt came with the higher productivity at the cost of the excessive use of fertilizers but gave rise to some negative ecological consequences also like water pollution and nutrient imbalance thus aptly referred to as "fatigue of green revolution". So, the great concern of the present day farm scientists should be to explore environmentally sustainable techniques and approach which can minimize the application of inorganic fertilizers without decreasing the performance of crops.

Equally important is the selection of the crop to be grown under such conditions and mustard fits into the plan as it is also an important plant which can remove some of heavy metals from the growth medium (Sharma, 2004). It may be of interest to note that rapeseed-mustard or brassicas are the most important among oilseed crops of the world after soybean and palm. Following the Mission Technology of Oilseeds 1986, the edible oil production was more than double in the next ten years (Bhatnagar *et al.*, 2001). India has diverse agroclimatic conditions favouring the cultivation of seven different rapeseed-mustard, namely Indian mustard, toria, yellow sarson, brown sarson, gobhi sarson, karan rai and taramira. Therefore, this crop ranks second after groundnut in area and production and is likely to become number one oilseed crop in the near future. This country has about 25% of world acerage and 14% of production ranking 4th in the world (Bojaria, 2000). Its oil has been a favourable cooking, preserving and pickling medium because of its pungency in addition to being used in massage, as an industrial lubricant and a base for polymer synthesis. The seed meal left after oil contains good amount of protein and is used in the form of oilcakes for livestock (Bhowmik, 2003).

Keeping the above mentioned facts in view, it was decided to study the feasibility of using the wastewater as a source of irrigation and nutrients together with various doses of NPK fertilizer for the cultivation of mustard so as to observe fertilizer economy and also to dispose off the wastewater through agriculture. The study comprised four experiments with the following aims:

1. To study the comparative effect of the raw wastewater, ground water and 50% diluted wastewater on the performance of *Brassica juncea* var. Varuna in the presence of graded doses of nitrogen, determining the optimum dose of nitrogen and the possibility of utilization of nitrogen and other nutrients present in the wastewater in view of nitrogen economy (Experiment I).
2. To determine the optimum dose and economy of phosphorus by growing the crop with various levels of phosphorus and the above (1) types of water (Experiment II).
3. To establish the best dose of potassium and also its economy by growing the crop with graded levels of potassium under the same types of water mentioned above (Experiment III).
4. To confirm the earlier findings on the same crop in presence of the optimum NPK doses obtained in experiments I-III alongwith the half of the optimum

dose to see if fertilizer saving could be achieved under the same waters while observing the interactions between fertilizer doses and wastewater (Experiment IV).

Thus, the main objectives of this study were to minimize the use of inorganic fertilizers and optimize the utilization of nutrients present in the wastewater by using it in crop cultivation, instead of throwing it in a water body and therefore, reducing the burden on the fresh water availability at least around urban areas of the country where this wastewater is easily available (Fig. 1).

Fig. 1(a) Drain showing the pumping of wastewater at farmer's field out side the city of Aligarh from where the wastewater was collected

Fig. 1(b) Another view of wastewater pumping at farmer's field of cabbage from where the wastewater was collected

REVIEW OF LITERATURE

Contents

REVIEW OF LITERATURE

2.1 Glossary of plant species cited in the text

Common Name/vernacular name	Botanical Name
Abutilon (Kanghi)/Flowering maple	*Abutilon indicum*
Alfalfa	*Medicago sativa*
Amaranth	*Amaranthus spinosus*
Ambavah	*Rumex dentatus*
Banana	*Musa paradisica*
Barley	*Hordeum vulgare*
Beetroots/Fodderbeets	*Beta vulgaris*
Bengal gram/Chickpea/Gram	*Cicer areitinum*
Black gram/Urd	*Phaseolus mungo*
Bottle gourd	*Lagenaria siceraria*
Broad beans	*Vicia faba*
Burmedagrass	*Cynodon dactylon*
Cabbage	*Brassica oleracea* var. *capitata*
Capsicum	*Capsicum frutescens*
Carnation	*Dianthus caryophyllatus*
Carrot	*Daucus carota*
Cassod tree	*Cassia siamea*
Castor bean	*Ricinus communis*
Cattail	*Typha angustata*
Cauliflower	*Brassica oleracea* var. *botrytis*
Celery	*Apium graveolens*
Chilli	*Capsicum annuum*
Chinese cabbage	*Brassica rapa* L. ssp *pekinensis*
Cluster bean	*Cyamopsis tetragonaloba*
Coffee weed	*Cassia occidentalis*
Coriander	*Coriandrum sativum*
Cotton (Upland Cotton/Kapas)	*Gossypium hirsutum*
Cowpea	*Vigna sinensis*
Cucumber	*Cucumis sativus*
Egg plant	*Solanum melongena*
Egyptian clover (Berseem)	*Trifolium alexandrinum*
Ethopian mustard	*Brassica carinata*
False rageweed	*Parthenium hysterophorus*
Fennel	*Foeniculum vulgare*
Fenugreek	*Trigonella foenum-graecum*
Finger millet	*Eleusine coracana*
Foetid	*Cassia tora*
Green gram (Moong)	*Phaseolus radiatus*
Groundnut	*Arachis hypogea*
Hemp	*Cannabis sativa*
Horntwort	*Ceratophyllum demersum*
Indian rosewood/Sheesham	*Dalbergia sissoo*
Kidney bean/Frenchbean/ stringbean/Bush bean	*Phaseolus vulgaris*

Common Name/vernacular name	**Botanical Name**
Lentil	*Lens culinaris*
Lettuce	*Lactuca sativa*
Leucerne	*Medicago sativa*
Linseed	*Linum usitatissimum*
Maize/Corn	*Zea mays*
Melon (Tarbooz)	*Citrullus vulgaris*
Minneola tangelo	*Citrus reticulata*
Motha/Nutsedge	*Cyperus rotundus*
Musk melon (Kharbooz)	*Cucumis melo*
Oats	*Avena sativa*
Oleander	*Nerium indicum*
Olive trees	*Olivia*
Onion	*Allium cepa*
Orange	*Citrus sinensis*
Parseley/Persele	*Apium petroselinum*
Pea	*Pisum sativum*
Pearl millet/Bajra	*Pennisetum typhoides*
Pigeon pea	*Cajanus cajan*
Potato	*Solanum tuberosum*
Prostrate spruge	*Euphorbia prostrata*
Radish	*Raphanus sativus*
Rape/Mustard/Winter rape/Spring rape	*Brassica juncea*
Red gum/Blue gum	*Eucalyptus*
Rice	*Oryza stiva*
Rose	*Rosa demascena*
Sesame/Gingelly	*Sesamum indicum*
Sorghum	*Sorghum vulgare*
Soybean	*Glycine max*
Spinach	*Spinacea oleracea*
Strawberries	*Fragaria vesca*
Sugarcane	*Saccharum officinarum*
Sunflower	*Helianthus annuus*
Tomato	*Lycopersicon esculentum*
Triticale	*TriticaleSecale Serele*
Tropical duckweed/Water lettuce	*Pistia stratoites*
Water hyacinth	*Eichhornia crassipes*
Water thyme	*Hydrilla verticillata*
Wheat	*Triticum aestivum*
White popinac/Horse tamarind	*Lucaenea leucocephala*

Chapter 2
REVIEW OF LITERATURE

2.2 Effect of Wastewater on Plants

Water pollution could have started with the establishment of human populations near the bank of rivers, streams and also lakes, seas and oceans. Initially these water bodies had a capacity to purify the wastes thrown in them, but due to increase in population, industrialization and urbanization they lost their carrying capacity and thus got deteriorated, which not only lead to the shortage of clean water but also affected their aesthetic value. From 19^{th} century itself fertilizer value of the wastewater especially of sewage has been recognized and the best way of its disposal was proposed to use it for irrigation taking into account its quality as one of the important parameters. In this review attempt has been made to include the literature related to the effect of wastewater on plants, use of NPK as fertilizers and heavy metals present in wastewater. It may also be pointed out that although some references related to wastewater were available, in the present review, only the studies undertaken since 1990 were included in this review.

Thus, Bahadur and Sharma (1990) from Bareily, while working on the effect of industrial effluent in relation to seed germination and seedling growth of wheat var. UP-115, reported that germination percentage decreased and the percentage of germination was maximum on the first day. Significant decrease was observed in root and shoot length and latter was much more affected than the root length. Goud *et al.* during the same year 1990 from Gujrat reported that dimethyl tetrapthalate industry wastewater had COD upto 80,000 mg l^{-1}, when this wastewater was mixed with bacterial culture of *Pseudomonas* sp., *Aeromonas* sp., *Arthobacter* sp. and *Bacillus* sp., COD and BOD were decreased upto 86% and 95% respectively in 48h. Germination tests with seeds of sorghum, green gram and millet indicated complete removal of toxic pollutants from the wastewater.

Jabeen and Saxena while observing the effect of Sarya distellery and Gorakhpur fertilizer factory effluents on pea in 1990 reported that 5% of distillery effluent and 2.5% of fertilizer factory effluent increased the dry matter, pigment and protein contents. They were of the opinion that both waters, if used after proper dilution, may be an additional source of nutrients. Subramani *et al.* (1990a) at Annamalainagar, while observing the effect of pre-sowing seed hardening treatment on the growth and yield of blackgram, also under distillery wastewater, reported that shoot length, root length, number of nodules, number of leaves, total leaf area and yield got reduced. Seed hardening with 1% $CaCl_2$ amended the adverse effect of wastewater by enhancing growth and yield parameters to certain extent. In 1990(b), while taking the green gram under the same effluent, they reported that lower concentrations were favourable where as higher inhibited the viability and percentage of germination. In continuation an attempt was also made by them in 1995(a) to study the effect of effluent on green gram and reported that it was highly acidic and when used at higher concentration showed deleterious effect on growth and productivity of crop. In 1995(b) they grew water hyacinth plants in raw distillery effluent for five days. Both raw and treated effluents were tested on green gram and seedlings grown at 5% and 10% concentration of biologically treated effluent showed increase in growth. They concluded that water hyacinth was efficient in removing the harmful effects of effluent. Again in 1999, Subramani *et al.* grew common hornwort for five days in distillery effluent and this effluent, when applied for the irrigation of green gram, promoted the growth and yield to some extent. Somashekar *et al.* (1992) at Bangalore (Karnataka), also working on the distillery wastewater, conducted laboratory experiments to study its effect on germination and growth of cowpea and fenugreek. The effluent was acidic and contained sufficient amount of dissolved solids, its oxygen saturation level was low and BOD and COD values were high. Effluent also contained significant amount of chlorides, potassium, sulphates and magnesium. Therefore, concentration of effluent had direct effect on rate and percentage of germination and plant biomass showed decreasing trend with the increase in concentration. Distillery effluents were also tested by Kannabiran and Pragasam (1993) at Pondicherry. They reported that blackgram seeds failed to germinate in undiluted effluents, but very few germinated in 75% effluent. The morphological and biochemical parameters had low values at 10, 25 and 50% concentration. The values were more or less similar in 1%, 5% and control, and 25%

proved optimum. Studies were also made by Goyal *et al.* (1995) at Hisar on distillery wastewater applying upto 160m^3 ha^{-1} to green gram which increased dry matter production and N and P uptake. However dry matter decreased markedly with 640 m^3 ha^{-1} application of wastewater. In 1998, Rajannan *et al.* at Coimbatore (Tamil Nadu) composted the press mud and distillery effluent and applied it to sugarcane. Addition of 2.5 t ha^{-1} effluent based compost improved the yield and also the soil fertility level, especially the K status. Singh and Bahadur during the same year at Pantnagar applied similar effluent as a pre-sowing irrigation on maize. Twelve pre-sowing treatments had no adverse effect on germination and improved the growth and yield. The pH and EC of soil got slightly increased where as soil organic carbon and NPK contents were increased significantly with the increase in the pre sowing effluent irrigation. In 2001, Kannan at Periyakulum Theni also applied distillery effluent in different concentrations to green gram and pearl millet. There was no germination in seeds irrigated with 100% effluent whereas irrigation with 1% effluent gave the highest values for germination percentage, shoot length, root length, and vigour index.

Similarly, Ramana *et al.* (2002a) at Bhopal (MP) also conducted a field experiment with groundnut to evaluate the potential of three distillery effluents, raw spent wash (RSW), biomethanated spent wash (BSW), lagoon spent wash (LSW) and NPK + FYM in addition to a control and found that all the three effluents increased the total chlorophyll content, crop growth rate, total dry matter, NPK uptake and the seed yield but inhibited the nodulation and decreased the nitrogen fixation. BSW produced seed yield double of control followed by RSW however, it did not affect the protein and oil content, thus concluded that these effluents can reduce the fertilizer requirement of crop. However, crop performance and yield with effluent was overall less than produced by NPK + FYM. In the same year Ramana *et al.* (b) while taking 0, 5, 10, 15, 20, 25, 50, 75, and 100% concentrations of RSW observed the effect on tomato, chilli, bottle gourd, cucumber, and onion and noted that 5% concentration was critical for seed germination in tomato and bottle gourd and 25% for other crops.

Sharma *et al.* (1990) while working on physico-chemical properties of Bhilai steel (MP) wastewater and its effect on soil and plant characteristics reported that water collected from 2 km downstream contained 2534, 5741, 6441 and 31 mgl^{-1} of

NO_3N, NH_4N, Fe and phenol respectively and when applied to linseed in the field and to sesame and French bean in pots decreased the plant Ca and Mg concentration while increased the P concentration. Fe was decreased in sesame and French bean, but increased in linseed. Soil nitrogen and sulphate were also increased while the effect on soil P, Mg, Fe, Cl and alkalinity varied with plant species. They suggested not to use this effluent for irrigation.

Misra and Behera (1991) at Berhampur working with paper industry effluent and rice cv Kesari-82k, in relation to concentration of effluent and time of exposure, reported that percentage of germination, water imbibing capacity, growth, pigment, carbohydrate and protein contents showed decreasing trend with the increase in concentration and time. Protein was the most sensitive macro molecule affected by the effluent. In 1991, Srivastava at Jabalpur (MP) also studied the effect of paper mill and chloro-alkali plant effluents on radish and onion seeds and reported that the latter was highly deleterious for germination and seedling growth as compared to the former. Similar, studies were also made by Agarwal and Chaturvedi (1995) at Faizabad on increasing concentration of paper mill effluents. An adverse effect on the amount of chlorophyll a, b and total chlorophyll of wheat was observed while Baruah and Das (1997) at Guwahati during their study on rice with similar effluent reported delay in germination and reduction in final germination percentage upto 12.5% as compared to control as effluent contained large amount of chloride, sulphate, carbonate, bicarbonate, magnesium, calcium and sodium.

In an experiment conducted by Dutta and Boissya (1997) it was noted that Nagaon paper mill effluent inhibited seed germination and seedling growth of rice at higher concentration. The seeds from effluent affected areas were also less viable and even viable seeds showed delayed germination in comparison to control. In 1999, Dutta further reported that the effluent was also harmful to the growth of paddy plants. During the same year Dutta and Boissya further noted that leaf area and chlorophyll content could not be directly co-related in both affected and non affected rice plants. Under similar conditions leaf area and total number of grains were directly proportional to each other in both affected and non affected areas when taken separately. In continuation to their earlier work, another experiment was carried out by them in 2000 to study the effect of the same effluent on flower number, number of fertilized flowers, average length of ear, number of grains, test weight, volume of 1000 grains and moisture percentage of fresh seeds of rice. They noted a significant reduction in each parameter compared to plants grown in area away from the effluents.

An experiment was also conducted by Dhevagi and Oblisami (2000) in Tamil Nadu to study the effect of similar effluent on various crops like maize, groundnut, sunflower, soybean, blackgram, green gram, sesame and castor beans. In their observations, raw effluent affected germination percentage of maize, groundnut, soybean and black gram and the highest reduction in vigour index was observed in castor bean and sesame.

Ramanujam from Pondicherry in year 1991 reported that concentration exceeding 10% of untreated sewage effluents reduced the germination percentage and also retarded the seedling growth of blackgram. Al-Jaloud *et al.* in Saudi Arabia during the year 1993 while irrigating maize and sorghum with sewage wastewater reported that crops showed significant improvement with the increase in water salinity presumably due to nutrients present in it. Plant yield however decreased slightly at water salinity level of 2330 mgl^{-1} TDS Overall soil salinity and sodicity had significantly greater effect on sorghum than on maize. Chakrabarati and Nashikkar (1994) at Nagpur while testing the wastewater containing sewage and sludge showed that water had generally favourble effect on germination and early seedling growth of forest tree sps. The effects of long-term irrigation with untreated sewage effluent on soil fertility and nutrient supply of alfalfa were studied in Mexico by Siebe (1998). Wastewater improved the nutrient balance of soil in respect to total nitrogen and available phosphorus. Calcium in alfalfa decreased, while phosphorus and sodium increased after several years of wastewater irrigation. Nancy Ebner *et al.* in 1999 from Chile also reported the suitability of sewage water which was used for irrigation of minneola tangelo and carnations. Treatment of tangelo with treated wastewater resulted in the improvement in nutrient status of soil and foliage, resulting in increased budding and flowering. Irrigation for carnation also proved beneficial. Reboll *et al.* (2000) at Castellon (Spain) applied wastewater from a sewage treatment plant on young citrus trees and observed no detrimental effect. More importantly fertilizer rates were also lowered significantly without decreasing the yields or affecting the nutrient levels. Impact of biologically treated sewage water was also studied on wheat and black gram under different fertilizer levels by Pradhan *et al.* (2001) at Pratapgarh. They did not found any significant difference in yield and its attributes of wheat. However, yield attributing characters like number of tillers plant^{-1}, panicle length, number of grains panicle^{-1} and 1000 grain weight of black gram showed increasing trend 13.29, 1.59, 5.49, and 3.79% respectively over tubewell irrigated plants. Yield was also increased to 3.63% when the crop was irrigated with sewage water. This water with $N_{60}P_{45}K_{45}$ responded better for tillering, panicle length, number of grains panicle^{-1} and yield of wheat. The same water showed negative effect on these parameters when applied on green gram. In Greece the effect of sewage sludge and waste water from a sewage treatment plant was studied on cotton by Tsakou *et al.* (2001). There were no significant differences among plant roots, stems or leaf surface of the same group due to irrigation water. The use of sewage resulted in significantly higher and more robust plants with faster development and greater stem and root biomass production. On the other hand irrigation with wastewater instead of tap water resulted in significant differences in root biomass and leaf surface in combination with high ratio of sewage sludge. Javid *et al.* (2003) while studying the effect of sewage wastewater on wheat reported that its application promoted growth and yield. Khan *et al.* (2003) also applied sewage wastewater mixed with industrial wastewater of Aligarh to spinach and fenugreek and reported that chlorophyll a, b, total chlorophyll content, photosynthetic rate, photosynthetic water use efficiency, growth and yield were increased. Shah *et al.* (2005) also from Aligarh reported that sewage wastewater proved better than ground water for growth, and productivity of triticale.

Salgare and Andhyarujina (1991) at Mumbai while observing the effect of polluted water from Patalganga river on mineral contents of its bank vegetation collected plants from three sites i.e. unaffected area of upstream, midway lowstream and highly polluted lowstream and noted that Na, K, Li, Ca, Mg, Fe, and P content of four species were inhibited while Cl was enhanced. Salgare and Acharekar (2000) further observed that industrial pollution of Kalu river inhibited the studied parameters of five weeds. While studying the effect of industrial pollution at Sewri, Salgare and Palathingal (2000) reported that in an unpolluted area germination was quick as compared to polluted area in cassod tree. However in another study on oleander conducted during 2001 they did not observe any significant difference in germination percentage between polluted and unpolluted areas.

Shukla and Pandey (1991) soaked the seeds of maize, black gram and chickpea in different concentrations of wastewater from oxalic acid manufacturing plant and reported that seed germination decreased from 100% in distilled water to 86, 32, 55% respectively in 25% concentration and 52, 12 and 15% respectively in 50% wasterwater. After 10 days, growth of seedlings under 25% was 5.1, 0.7 and 2.5 cm more compared to control. Trivedi and Kirpekar (1991) at Karad (Maharashtra) carried out an experiment to study the impact of dairy wastewater irrigation on growth and mineral composition of soybean and blackgram and reported that it increased Ca, N, P in both crops. They also observed that P contents were increased in 10%, 25% and 50% but decreased in 100%. Studies were also conducted by Prasanna *et al.* (1997) at Bhavanagar on the effect of dairy effluent on green gram and black gram. Seeds were soaked in 0, 25, 50, 75 and 100% concentration and they found a gradual decrease in seed germination, seedling growth and pigment content with increase in concentration. For all parameters, 25% proved better and concluded that the effluent can be safely used for irrigation if diluted to 25%.

Abasheeva and Revenskii (1992) in Russia applied clear water or purified wastewater from a cellulose and cardboard mill containing 1 gm salt l^{-1} to oats, rape, and peas grown in alluvial meadow or grey forest soil and observed that wastewater increased dry matter yields of oats on both the soils and peas on grey forest soil. There was no effect on rape on either of the soils or peas on alluvial meadows.

Cluster beans were grown with phosphatic fertilizer factory effluent at Raipur (MP) by Goswami and Naik (1992) and it was reported that effluent was extremely acidic in nature, had higher concentration of total dissolved solids, fluoride and sulphate, therefore higher concentration adversely affected the chlorophyll content, however 10% concentration improved it. While textile effluent was studied by Gupta and Nathawat (1992) in Rajasthan and they reported toxic effect on seed germination and seedling growth of pea. With the increase in concentration of effluent, there was a decrease in root and shoot length and total biomass. Root length was more adversely affected than shoot length. In 1992 also, Pathak *et al.* at Bhavanagar (Gujrat) carried out an experiment on utilization of effluent of Excel India Ltd for agrochemistry. They reported that white popinac grow well on soil irrigated with effluent.

At Aligarh much work has been undertaken on the effect of wastewater of thermal power plant, refinery and sewage wastewater on various crops. Aziz *et al.* (1993a) carried out a field study on the effect of treated refinery wastewater on nitrate reductase activity of green gram var. T44 and K851 and reported that enzyme was stimulated more by effluent application in the former variety. In another study (1993b) the same effluent proved beneficial for growth and yield parameters of lentil. Another field study was conducted by Aziz *et al.* (1994) to evaluate three cultivars of triticale and one of wheat. The refinery effluent increased growth and yield parameters. Soil irrigated with effluent showed no significant change in pH, total organic carbon, calcium, water soluble salts, CEC, and SAR. They further reported that triticale performed better than wheat under wastewater. Performance of industrial wastewater mixed with sewage wastewater on wheat, mustard, cauliflower, spinach, cucumber and agricultural soil was observed by Aziz and Inam (1995). They reported that soil showed no significant change in pH, EC, organic carbon and some cations but the accumulation of heavy metals like Pb, Cr and Ni was shown by the soil and crops grown in it. Leaves of all crops accumulated more heavy metals and inorganic chemical constituents as compared to grains, stems and roots of the crops tested. In 1995, Aziz *et al.* also observed improvement in growth and yield characteristics of four cultivars of wheat. In 1996a, Aziz *et al.* in another study reported that crops like wheat, triticale, chickpea, lentil and pigeon pea,but no summer moong,can be grown under refinery treated water. During the same year Aziz *et al.* (1996b) observed increase in fresh yield of berseem, although the same water affected adversely other leguminous crop in their earlier study (Siddiqi *et al.*, 1994) conducted on moong. Aziz *et al* (1998). conducted a split plot field experiment again on triticale under five levels of irrigations. They noted that treated effluent had more nutrients available as compared to ground water. Therefore, a linear increase in growth and yield was observed with the increase in frequency of irrigation upto three, which even proved superior to four irrigations of ground water. Aziz *et al.* (1999) also cultivated maize and mustard and noted improved growth under this water. Inam *et al.* (1993) observing refinery effluent on seedling emergence of triticale and wheat reported no adverse effect and Samiullah *et al.* (1994) also found the effluent beneficial for wheat. Also at Aligarh an experiment was conducted by Saeed *et al.* (2003) to evaluate the utility of thermal power plant wastewater for green gram which exhibited better response in terms of growth and yield.

At Sivakasi, Ramasubramanian *et al.* (1993) soaked the seeds of black gram in diluted effluent (10 to 40% v/v) of match and dye industry and grew them in sand culture. They observed decrease in germination percentage and seedling length with increase in concentration. Decrease in fresh and dry weight paralleled a decrease in leaf pigments. Further, leaf soluble protein and in vivo nitrate reductase activity decreased and leaf L-proline increased as the concentration of effluents increased. Vijayakumari *et al.* (1993) at Coimbatore tested different effluent of soap factory on millet and pulse crops and observed that 2.5% and 5.0% promoted the seedling growth of finger millet while 5.0% effluent enhanced the seed germination and seedling growth to maximum extent in pearl millet. 25% enhanced overall growth of green gram and black gram seedlings. However, 100% inhibited completely the seed germination as well as seedling growth of both crops.

Different concentrations, ranging from 2.5% to 100% of tannery effluent, were applied on green gram, pigeon pea, and sorghum by Balashouri and Prameeladevi (1994). Seed germination, seedling growth, chlorophyll content and biomass accumulation were generally optimum in legumes at 10% and in sorghum at 5% effluent. Higher concentrations had inhibitory effect. Karunyal *et al.* (1994) at Madurai also applied tannery effluent (25, 50, 75 and 100%) on rice, abutilon and white popinac and noted that germination was inhibited by 25% and 50% but prevented by 75% and 100% effluent. Leaf area, biomass and chlorophyll content of cotton, black gram, cowpea and tomato of effluent treated plants were examined. Leaf area and biomass of 25% effluent treated plants showed an increase over control and same was the case with total protein and chlorophyll contents. They recommended 25%, suitable concentration as 75% and 100% killed the plants.

Arora and Chauhan (1996) at Agra also studied the tannery effluent and observed significant reduction in germination percentage and length of almost all varieties of barley they studied. Bera and Saha (1998) at Mohanpur while applying similar effluent at 1, 2.5, 5, 10, 25, and 50% to pigeon pea and rice reported that seedling growth was stimulated under 10% and 5% respectively. From the same place Bera and Bokaria (1999) reported that at 50% concentration, germination was 64% compared to 96% in control. Early seedling growth, dry weight and fresh weight of green gram were better at 2.5%.

While irrigating crops with a mixture of industrial discharge and rainfall Jimenez *et al.* (1995) from Mexico reported that crop yields were about at national average level for maize and leucerne. During the same year, Sawarkar *et al.* (1995) at Jabalpur in pot experiment applied phosphorus 0-45 ppm and S as oxalic acid industrial wastewater between 0-150 ppm to Indian mustard. They observed an increase in seed yield with increase in P rate and also with 100 ppm S. Phosphorus application also increased seed P and oil content. Sulphur application increased S content and available S in soil. At Shillong, seeds of chickpea, black gram, maize and rice were sown by Shukla and Moitra (1995) in 0, 25, 50, 75 and 100% concentrations of steel plant effluent. Seed germination and seedling growth of all crops were decreased with the increase in concentration and the lowest tolerance was shown by maize.

At Guntur, Umamaheshwara and Rao (1995) studied the effluent from thermal power station. Sulphur and chlorophyll contents of the leaves of surrounding vegetation were found to be higher in tested plants. However, no adverse effect on vegetation was recorded. Srivastava (1996) also from Guntur reported that effluents from industries like sugar and distilleries containe higher amount of phosphate, sulphate, calcium, ammonia, total N and have more BOD, COD, conductivity, temperature and turbidity, less amount of dissolved carbon and total absence of DO. However, crops treated with diluted effluents showed moderate tolerance and better biomass as compared to concentrated effluents.

Iqbal and Mehta (1998) at Jaora (M.P.) reported that sugar mill effluent increased the dry matter in wheat, where as chlorophyll content and dry matter of chickpea was adversely affected. In 2000, Kumar from Bihar while observing the influence of periodic watering with Chakia sugar mill effluent on barley reported reduction in growth and yield parameters and suggested the employment of proper remedial measures to minimize the pollution load of the effluent before its disposal. While working on industrial wastewater of Kattedan industrial developmental area, Prashanthi and Rao (1998) reported that it was unsuitable for the germination of crops. Also in 1998, Vassilev *et al.* at Granada (Spain) tested microbially treated olivemill wastewater (OMW) with added N and Mg as a medium for *Aspergillus niger*. The latter was able to reduce the phenolic component of waste material to 59% of its initial amount and to lower the pH of the medium. Rock phosphate (RP) added to this water was solublized to its maximum amount. Five types of OMW+RP microbially treated or not were tested in a soil trifolium system for their fertilizing ability. The best plant growth response and P uptake were found in microrrhizal soil amended with fungal treated OMW+RP.

From Lebanon, Darwish *et al.* (1999) recommended the reuse of secondary treated wastewater for the production of banana, leucerne, tomato, cucumber, melon, capsicum, rose, and carnation under three conditions. First represented optimally under existing cropping pattern while in second and third new crops were introduced. Lowest net return was obtained under first condition, where as higher profits were achieved under the other two. Ponmurugan and Jayaseelan during the same year at Sivakasi observed that higher concentrations of fire works and dye industrial effluents were responsible for decreasing the germination percentage, reduction in root and shoot morphology, phytomass and biomass accumulation of cattail. Srivastava and Pandey (1999) at Faizabad reported that total chlorophyll content and biomass of water hyacinth, water lettuce and water thyme were reduced significantly with increase in treatment durations from 7, 14 and 21 days of exposure to different fertilizer factory effluent concentrations.

The influence of industrial effluent of sugar factory and Aska-chloro-alkali factory at Ganjam district on degradation of photosynthetic pigment in intact paddy leaves was determined by Balaram *et al.* (2000). They found that Aska effluent was more effective at all concentrations in degrading the chlorophyll content and Rayagada effluent was least effective. Baumgartel and Fricke (2000) in Germany carried out studies to monitor the soil nitrogen levels during autumn-winter after the application of wastewater from starch potato factories in late summer. The uptake of N and K by mustard, green manures, cover and catch crops was monitored and finally recommended its application. Kumar *et al.* (2000) at Saharanpur studied the effect of polluted water of Hindon river on plant distribution on the river. Results revealed that amaranth, false rageweed, hemp and ambavah showed tolerance to polluted water where as nutsedge and prostrate spruge showed higher density along the clean river water side. In Spain, Murillo *et al.* (2000) conducted an experiment to examine the effect of drip irrigation while using wastewater from a table olive industry on olive trees. They tested two types of water, one with SAR and EC value of 12-56 and 3.5-4.2 ds m^{-1} and the other with 73-90 and 4.3-6.0 ds m^{-1} and both significantly reduced the yield.

Augusthy and Mani (2001) at Arunapuram reported that rubber factory effluent has high amount of TSS, TDS, sulphate, phosphate and total nitrogen, so the effluent diluted upto 50% favoured seedling growth, root length, shoot length and number of lateral roots. However seed germination percentage of green gram was retarded in higher concentration While studying the effect of dye and printing industry effluent on chickpea and wheat, Kumawat *et al.* (2001) at Ujjain reported that effluent did not show any adverse effect on germination and growth of the crops tested under lower concentrations. However they were of the opinion that proper crop cultivar selection is important. Mathusamy and Jayabalan (2001) at Tiruchirappalli applied 0, 25, 50, 75 and 100% concentration of sago and sugar factory effluent on cotton var. MCUS and MCU 11.25% of sugar factory effluent had stimulatory effect on biochemical contents observed, but all concentrations of sago factory were having inhibitory effect except on proline content, which increased with increasing concentration of both the effluents.

In a two year study conducted by Adeli *et al.* (2003) reported that application of swine lagoon effluent irrigation increased the dry matter production of burmedagrass. Beneficial effect of thermal power plant wastewater and sewage water on linseed and black gram was also reported at Aligarh by Akhtar *et al.* (2006) and Javid *et al.* (2006) respectively. Distillery effluent was also tested by Khan *et al.* (2006) at Ghaziabad for the cultivation of sugarcane. Increase in germination percentage, number of nodes and internodes, girth, height, millable cane per clump, chlorophyll content and yield over the plants grown in control conditions was observed by them.

In addition beneficial as well as harmful effect of various types of wastewaters (paper mill effluent, distillery effluent, industrial effluent, tannery effluent, chemical industry effluent, olive mill effluent, Hindustan newsprint factory effluent, foam factory effluent) on number of crops (eucalyptus, wheat, maize, cabbage, lettuce, parsley, onion, carrot, fennel, radish, tomato, rice, mustard, lentil, green gram, chickpea, blackgram, pigeon pea, soybean, egg plant, olive trees, foetid, coffeweed, broad bean and burmedagrass) have also been observed elsewhere from time to time. Mention may be made of, Veena *et al.* (1992) Lagacherie *et al.* (1993). Armon *et al.* (1994), Saha *et al.* (1994), Singh and Bahadur (1995), Chidaunpalan *et al.* (1996), Khan and Srivastava (1996), Sujatha and Gupta (1996), Al-Nakshabandi *et al.* (1997), Ceggara *et al.* (1997), Jabeen and Abraham (1997), Barman *et al.* (1999), Mahankale and Dauore (2001), Rampal and Dorjey (2001), Crowe *et al.* (2002) and Heaton *et al.* (2002).

2.3 NPK Fertilizers and Mustard

Among the macro essential nutrients N, P and K are consumed in larger amounts, as they are removed by crops in large quantities. Like other crops mustard also showed good response to these nutrients as may be observed under the references reviewed here. It may also be pointed out that references on mustard grown under wastewater were few as explained earlier, therefore, this part of review is based on NPK fertilizers only and that toofrom 1990 onwards.

Saran and Giri (1990) conducted field experiments at IARI, New Delhi to study the effect of nitrogen, phosphorus and sulphur under semi-arid conditions. Results revealed that 40kg N + 11kg P ha^{-1} increased the seed yield significantly.

Additional 40kg N ha^{-1} and application upto 60kg S ha^{-1} also increased the seed yield. Significant increase in the oil content was also recorded under 60 kg S ha^{-1}.

From Udaipur, Agarwal and Gupta (1991) reported that two irrigations resulted in significantly higher yield than one and no irrigation and was at par with three irrigations. 30kg ha^{-1} P_2O_5 significantly increased the plant height, siliquae $plant^{-1}$, seeds $siliquae^{-1}$ and seed and oil yield. Application of 60kg N ha^{-1} also increased the seed yield, its attributes and oil yield, however oil content was adversely affected. In 1991 Joshi *et al.* at Navasari (Gujrat) observed that 60 kg N ha^{-1} significantly increased the plant height, leaf area index, primary and secondary branches, siliquae $plant^{-1}$, seeds $siliquae^{-1}$, while 75 kg ha^{-1} sulphur application had no effect on the growth and yield attributes including the seed yield but markedly increased the oil content upto 34.69%. In the same year, Parihar at Kharagpur reported that grain yield and yield components of mustard were greater when irrigation was applied at irrigation depth cumulative pan evaporation (ID: CPE) of 0.6 alongwith 60kg N ha^{-1}. Frequent irrigation under 0.8 ID: CPE did not significantly increase either grain yield or water use efficiency over 0.6 ID: CPE. Significant increase in grain yield was observed with 60kg N ha^{-1}. Rana *et al.* from UP in 1991 reported negative effect of irrigation and nitrogen application on oil content, but oil yield was increased. Significantly higher content and uptake of N was obtained by irrigation at 0.6 IW: CPE and application of nitrogen @ 150 kg ha^{-1}. From Varanasi (UP) Singh *et al.* (1991) while studying the effect of nitrogen and phosphorus under rainfed conditions and 0, 25, 50 and 75kg N ha^{-1} and 0, 20 and 40kg P ha^{-1} in two experiments noted that increased levels of N increased significantly plant height, number of branches, siliquae $plant^{-1}$, seeds $siliquae^{-1}$, 1000 seed weight and seed yield. Maximum seed yield was recorded with 75 kg N ha^{-1} and oil content of seed decreased with every increase in N from 0 to 75 kg ha^{-1}.

Mohan and Sharma (1992) at Sabaur (Bihar) reported, from a two year experimental study, that plant height, secondary branches and dry matter $plant^{-1}$ increased significantly upto 75kg N ha^{-1} and functional leaves, leaf area index and primary branches upto 100kg N ha^{-1}. Sulphur at the rate of 75kg ha^{-1} also increased functional leaves, primary and secondary branches in both years and leaf area index during first year. Sulphur @ 50kg ha^{-1} increased plant height and dry matter production for both the years. Nitrogen @ 75kg ha^{-1} + 50kg S ha^{-1} significantly increased the number and size of siliquae, 1000 seed weight and also gave the highest seed yield on pooled basis. At Karnal, Singh *et al.* (1992) studied the effect of three subsurface drainage spacings and three levels of phosphorus on yield, chemical composition and uptake of nutrients. The number of siliquae m^{-2} and seed yield decreased under increased drainage spacing. Application of phosphorus increased the seed yield and its attributes. The concentration of N, P and K in seed and stalks decreased and those of Na, Ca, and Mg increased with increasing drain spacing, but phosphorus increased the concentration of these nutrients in seeds and stalks.

In 1992, field experiments were conducted by Tomer *et al.* at Hardwar (UP) to study the effect of irrigation and N, P and K. They noted significant increase in growth and yield with the increase in irrigation level and NPK, while oil content in seeds was decreased, where as protein content was unaffected upto two irrigations. Number of branches, dry matter accumulation and seed yield were maximum with 120, 60, 60 kg N, P_2O_5, K_2O ha^{-1} and two irrigations, while oil content was higher under no irrigation and fertilization.

In 1993, Prasad and Shukla from Varanasi reported that 30kg N ha^{-1} in combination with 60kg K ha^{-1} increased the plant height, number of leaves, number of branches and leaf area. They obtained grain yield upto 22.17 q ha^{-1} and 20.93 q ha^{-1} in two subsequent years. In the same year Vyas and Rai at IARI, New Delhi conducted a two year study and reported that mustard yield increased significantly with each level of phosphorus application from 0, 10, 20, 30kg P ha^{-1}. Similar results were also obtained with chickpea, however maximum equivalent yield for mustard was obtained with the application of 30 kg P ha^{-1}.

In 1994, at Aligarh, Mohammad reported that spray treatment with three different sources of phosphorus i.e. sodium dihydrogen orthophosphate, diammonium phosphate and monocalcium superphosphate proved significantly efficacious in augmenting most of the yield parameters, and oil yield was also increased by 20% over control. He further reported that all the three sources of phosphorus proved equally effective.

Results of the field experiment carried out by Saraswathy and Dharmalingam (1994) at Coimbatore (Tamil Nadu) revealed after giving 30, 40, 50kg N ha^{-1} and 10, 20, 30kg K ha^{-1} and a single dose of phosphorus 20 kg ha^{-1}, a combination of 50:20:30 kg ha^{-1} NPK proved superior by augmenting seed yield and quality of resultant seeds. At Hisar (Haryana) Yadav *et al.* (1994) noted that increase in the amount of water and N fertilizer increased the leaf water potential, stomatal conductance, light absorption, leaf area index, seed yield and evapotranspiration and decreased the canopy temperature. The combination of 0.6 ID:CPE with 60 kg N ha^{-1} gave significantly higher yield than lower rates and equalled to highest irrigation and N treatment combination.

In 1995, Chauhan *et al.* at Gurgaon (Haryana) worked on the effect of biofertilizer, gypsum and nitrogen. Results revealed that inoculation with *Azotobacter* and *Azospirillum* significantly increased the seed yield over no inoculation. Application of 125 kg gypsum ha^{-1} favoured the development of yield attributes and consequently increased the seed yield also. Nitrogen @ 80kg ha^{-1} recorded higher values of growth and seed yield over 60kg N ha^{-1}. Oil content was however adversely affected under higher nitrogen level. At IARI, New Delhi. Parmanik *et al.* (1995) reported that yield attributes, seed yield and oil yield increased significantly upto 100kg N ha^{-1} but harvested biomass upto 150kg N ha^{-1}. Irrigation and N interacted significantly for plant height, total branches and siliquae plant^{-1}, harvested biological and seed yield. Significantly higher seed production was obtained from the crop irrigated at IW:CPE ratio of 0.6 fertilized with 100kg N ha^{-1} but biomass was highest with 150kg N ha^{-1} at an IW:CPE of 0.8.

While applying 0, 50 and 100kg N ha^{-1} and 0, 40, 80kg P ha^{-1} to four varieties of mustard, Arthamwar *et al.* (1996) at Parbhani (Maharashtra) reported that 'Pusa Bold' recorded significantly higher values for yield attributes, seed and oil yield than other three varieties. During the first year N_{50} and N_{100} were at par and significantly superior to N_0, but during the second year they reported a linear increase from N_0 to N_{100} kg ha^{-1}. Nitrogen did not influence oil content whereas increase in phosphorus level significantly improved all the yield attributes, seed yield, oil content and oil yield.

In 1996, Hayat *et al.* also at Aligarh studied the response of mustard to nitrogen and phosphorus and noted that number of pods, number of seeds, seed-yield, oil yield, and N, P, K content were significantly increased. However, acid, iodine and saponification values were decreased over control. The basal dose of 80 kg N ha^{-1} and 30 kg K ha^{-1} gave the optimum performance. During 1997, Khafi *et al.* from Jamnagar (Gujrat) conducted an experiment to resolve the response of Kranti variety to nitrogen, phosphorus and foliar applied agrochemicals. Results revealed that nitrogen significantly increased the yield attributes and yield upto 80 kg ha^{-1}. Similarly application of 30 kg P_2O_5 ha^{-1} significantly increased the yield attributes, seed and stover yields. Foliar spray of sulphuric acid and thiourea also influenced the yield parameters and yield. Kumar *et al.* (1997) at Ludhiana while studying the leaf area index (LAI) relationship with solar radiation interception (SRI) and yield as influenced by the plant population and nitrogen noted that LAI, SRI and dry matter yield were obtained with 150kg N ha^{-1} and plant density of 444000 plants ha^{-1} at all the crop phenophases. The highest seed yield was obtained with 125kg N ha^{-1} and a plant density of 266000 to 333000 plants ha^{-1}. Sharma *et al.* (1997) at Jorhat studied the effect of crop geometry and nitrogen and reported that crop geometry had no significant effect while nitrogen treatment significantly increased the seed yield and its attributes.

While working on the effect of nitrogen and phosphorus on growth and yield of mustard and chickpea in intercropping, Singh *et al.* (1997) at Bulandshahar reported that Indian mustard grown as sole or intercropping responded upto 80 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1}, however economic dose of chickpea was 49.8 kg P_2O_5 ha^{-1}. While the seed yield remained unchanged due to nitrogen application in two years. In 1997, Tomer *et al.* conducted a two year field study at Gurukul Narsan to observe the effect of nitrogen, phosphorus and sulphur fertilization and found that growth and yield attributes increased significantly upto 180, 80 and 80 kg ha^{-1} respectively. The oil content of seeds decreased with increasing levels of N and P where as increased with increasing levels of S.

To assess the growth and yield in relation to sulphur and nitrogen interaction, Ahmad *et al.* (1998) at New Delhi reported maximum response with S_{40} and N_{100} (kg

ha^{-1}) however, oil percentage was maximum at N_{100} and S_{60}. Favourable response of S and N interaction on LAI, P_N and biomass was also observed. A field trial conducted at Parbhani by Patel and Shelke (1998) revealed that growth, yield attributes, seed as well as stover yield were increased significantly with the application of farmyard manure applied @ 5 tonnes ha^{-1} over control. Phosphorus upto 80 kg ha^{-1} showed linear increase in all the parameters, however during the first year primary branches, number of siliquae and during the second year, seeds and oil content, and in both the years plant height, leaf area, total dry matter and length of siliquae showed linear response upto 120kg P_2O_5 ha^{-1}. Similarly all these parameters increased significantly with increasing levels of sulphur upto 60 kg ha^{-1}, however significant effect on seed and stover yield was noted upto 30 kg S ha^{-1}. In the same year a field study conducted by Patel and Thakur at Raigarh revealed that seed, oil and protein yield was found significantly higher with the application of 60 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1} over 20 and 40 kg N ha^{-1} and 20 kg P_2O_5 ha^{-1} respectively. Further increase above these doses did not bring significant increase.

In 1999, Masthan *et al.*, carried out an investigation at Hyderabad to find out the dose of phosphorus sufficient to meet the nutritional requirement of rice, rapeseed and green gram cropping sequence. The concentration of N and K reduced by the application of 26.2 kg P ha^{-1} in rice and rapeseed among the three crops. The P concentration increased in rice and green gram but not in rapessed. The uptake of N, P, K was more in rice and rapeseed fertilized with 26.2 kg P ha^{-1}. Green gram in summer removed more NPK. Phosphorus @ 26.2 kg ha^{-1} to rice significantly increased the yield and its residual effect was also significant in increasing the production of rapeseed grown as second crop. The cumulative effect of residual P through previous crop of rice and its direct application both at 26.2 kg ha^{-1} P maximized the grain yield of rapeseed. During the same year Puri *et al.* at Jabalpur reported that highest seed yield and oil content were noted in treatment 100:40:20 NPK kg ha^{-1}. Significant negative correlation coefficient of oil content with seed protein content, with seed yield, seed S content and seed Mg content were also obtained. An experiment was conducted by Sharma *et al.* (1999) at Morena to study the effect of organic and inorganic sources of plant nutrients on productivity, nutrient uptake and economics of soybean and mustard. They reported that the highest seed yield for soybean was recorded with N_{40} (½N basal + ½ N top dressed) P_{80} and K_{20} kg ha^{-1}, where as in mustard yield was highest with N_{40} (½N through urea + ½ N through farm yard manure) P_{80} and K_{20} kg ha^{-1}.

A field experiment was conducted by Bhari *et al.* (2000) at Rajasthan Agriculture University to study the effect of 30, 60, 90 and 120kg N ha^{-1} and 0, 15, 30, 45kg P_2O_5 ha^{-1}. Application of N upto 120kg ha^{-1} resulted in significant increase in plant height, primary and secondary branches and siliquae $plant^{-1}$ except for seeds $siliquae^{-1}$ and 1000 seed weight upto 90kg N ha^{-1}, while 45kg P_2O_5 ha^{-1} resulted in more plant height, secondary branches and siliquae $plant^{-1}$. With the increased supply of phosphorus upto 45kg P_2O_5 ha^{-1} the N need of crop was raised upto 120kg ha^{-1} for higher seed yield.

While studying the effect of irrigation and nitrogen on the performance of mustard and sunflower, Giri (2001) at IARI, New Delhi reported that Indian mustard out yielded sunflower. Higher levels of irrigation and nitrogen did not bring significant improvement in the yield over lower levels. At Bhopal, Hati *et al.* in the same year while conducting a field study to assess the effect of irrigation levels and combination of NPK and farm yard manure on moisture use, evapotranspiration, water use efficiency and yield noted that moisture extraction was maximum from upper 0-30 cm soil depth irrespective of irrigation and nutrient treatment. Evapotranspiration increased with increasing levels of irrigation and reached its peak between 75-90 DAS. Seed yield increased significantly with increase in irrigation number but WUE was highest at no irrigation, followed by one and two irrigations respectively. Application of recommended dose of NPK (60, 13.1, 16.6 kg ha^{-1}) + FYM @ 10 tonnes ha^{-1} resulted in significantly higher yield. A study conducted by Hatmode *et al.* at Nagpur in the year 2001 revealed that 60:50 kg N and P_2O_5 ha^{-1} significantly increased the leaf chlorophyll, N and K content, number of siliquae $plant^{-1}$, seeds $siliquae^{-1}$, harvest index and seed yield. While oil content was significantly maximum at 40:30 kg N and P_2O_5 ha^{-1}.

Effect of irrigation schedules and fertilizers on growth, yield and quality was also studied by Pawar *et al.* (2001) at Parbhani. They noted that irrigation schedules at

0.6 IW:CPE ratio significantly influenced dry matter production, secondary branches and number of developed siliquae plant^{-1}. Maximum seed yield was recorded with 50kg N + 40 kg P_2O_5 + 40 kg S ha^{-1}. While working at Hisar, Punia *et al.* during the same year reported that plant height and siliquae plant^{-1} were significantly higher at a row space of 60 cm × 10 cm, but seed yield was highest under 30 cm × 10 cm spacing. They also observed significant response of nitrogen on seed yield, plant height and siliquae plant^{-1} at successive levels of N upto 80 kg ha^{-1}. Oil percentage decreased significantly with each successive increase in N dose upto 90 kg ha^{-1}.

In 2002, Kumar *et al.* at Ludhiana while studying the effect of nitrogen and sowing dates to four levels of N (0, 75, 100 and 125 kg ha^{-1}) under three sowing dates (21 October, 15 November, 10 December) noted higher productivity of rape sown on 21 October and 15 November over 10 December. Growth and yield characters were also significantly higher in 21 October and 15 November sown crop. The seed yield and oil yields increased significantly with each successive increase in N level upto 100 kg ha^{-1}. Interaction effect revealed that crops sown on 21 October, 15 November and 10 December responded to N applied upto 125, 100 and 75 kg ha^{-1} respectively. Shukla *et al.* (2002) at Pantnagar observed best morphological and physiological determinants along with highest seed yield and yield contributing characters at 100% recommended fertilizer + farmyard manure + S + Zn + B + Azotobacter, which was at par with 50% recommended fertilizers at same levels of supplementary nutrient management practice. At the same place Singh *et al.* (2002) while working on different fertility levels reported that branches plant^{-1}, seed weight plant^{-1}, 1000 seed weight, seeds siliquae^{-1} and seed yield were highest in PBC-9221 (Kiran) of Ethopian mustard. Oil content of Indian mustard was higher but oil yield was higher in PBC 9221, variety of Ethopian mustard. DLSC1 had higher FUE and due to increase in fertility levels upto 125% FUE increased with increase in fertilizer application, oil content decreased and contrary to it oil yield increased.

Chandrasekaran at Coimbatore (2003) worked on the impact of intercropping systems and 0, 20, 40 kg ha^{-1} of nitrogen levels on physiological parameters and yield of mustard, Bengalgram and coriander. Their result showed that sole cropping of mustard recorded higher CGR, NAR and seed yield compared to Bengalgram and coriander intercropping system. Nitrogen @ 40 kg ha^{-1} resulted in maximum CGR, RGR and NAR and yield of mustard and coriander where as 20 kg N ha^{-1} was sufficient for Bengalgram. In a field study conducted in the same year at Udaipur by Meena and Sumeriya also assessed this crop and reported that highest number of secondary branches was under two irrigations in combination with one interculture. Application of 30 kg N ha^{-1} gave significantly highest recovery of applied nitrogen under no interculture. They also observed that application of 60 kg N and 90 kg N ha^{-1} gave maximum oil and protein content respectively. A two year experimental study was also conducted by Singh and Prasad (2003) at Kanpur to study the effect of row spacing, nitrogen levels and basis of N application. They noted that all yield attributing characters were superior at 60 cm spacing and N_{120} performed better over N_{60} and N_{80}.

More recently Pandey and Bharati (2005) at Samastipur studied the response of NPK. Application of 90 kg N and 60 kg P and 60 kg K ha^{-1} recorded higher values of yield indices, produced significantly higher grain and stalk yields and enhanced NPK uptake. Oil content decreased by increased levels of nitrogen and not significantly influenced by the level of phosphorus and potassium. However oil yield increased significantly with the increasing levels of nitrogen, phosphorus and potassium. In a field experiment Premi *et al.* (2005) at Bharatpur while studying the effect of organic and inorganic nutrients reported that application of vermicompost @ 5.0 t ha^{-1} + 75% recommended dose of fertilizer recorded maximum plant height, number of primary and secondary branches, number of siliquae and number of seeds, which in turn resulted in higher seed yield. It was at par with farmyard manure @ 10 t ha^{-1} + 75% recommended dose of fertilizer.

2.4 Heavy Metals in Plants

Various elements including heavy metals are present in natural water but in wastewater their concentration may exceed many times of their normal concentration. Both phytoplanktons and vascular plants may concentrate these elements, which if exceeds certain limits may become toxic to plants and their consumers. In the following pages some related references on heavy metals in relation to plants irrigated with waste water have been reviewed briefly.

Truby and Raba (1990) at Freiburg (Germany) conducted a study of heavy metal uptake by garden plants under sewage farm wastewater and reported that it was responsible for the enrichment of heavy metals in soil and lead to increased uptake of Zn, Cd and Pb by Chinese cabbages, lettuce, fodder beets, spinach, cabbage, carrots, potatoes, radish, beet roots, celery, onion, bush beans, strawberries, cucumber and tomatoes. Low levels of heavy metals were found in vegetables and strawberries but in leaf and root vegetables Zn and Cd levels were generally high and exceeded statutory limits in most of the cases. However, lettuce, fodder beets, spinach, celery and carrots grown in neighbouring uncontaminated field also had high Cd contents which was responsible for poor conditions of plant growth.

In 1994, Barman and Lal at Lucknow (UP) reported that the levels of Zn, Cu, Cd and Pb in cultivation fields adjacent to Durgapur industrial belt were found to be much higher than background level. Bioaccumulation of these metals in different parts of 14 plant species was found either within or beyond the critical concentration and maximum localisation was found in edible parts followed by leaves and shoots.

In 1995, Sharma and Habib from Bareilly reported that rubber factory effluent had high magnitude of pollution due to Cr, Pb, Zn, Fe, Na, K, Ca, Mg, So_4 and Po_4. Different cultivars of wheat, chickpea, pea, mustard and barley were tested. Concentration of Mg decreased in the straw and dried hay of all the cultivars irrigated with effluent water. Bioaccumulation of Pb, Cr and Zn differed in cultivars. Percentage of Ca, K, Pb, PO_4, total N and crude protein was lower in the seeds of effluent treated cultivars of chickpea. On the contrary Na, Fe, So_4 total carbohydrates and Cl increased significantly. Eid and Sherief (1996) at Cairo (Egypt) irrigated barley, broad beans and rape under green house conditions with wastewater. The treatments were (1) raw wastewater mixed with fresh water in the ratio of 1:2 for EC of 5ms cm^{-1} (2) raw wastewater mixed with fresh water in the ratio of 1:6 for EC of 2ms cm^{-1} (3) and treated wastewater mixed with fresh water in the ratio of 1:6. Highest dry matter yield was recorded with the last treatment where as P, N, Mn and Ni contents increased in plants after irrigation with mixed wastewater in comparison to fresh water. NPK contents were more in grain or seed but Fe and Mn contents were more in straw.

Fazeli *et al.* (1998) at Mysore reported that irrigation of paddy plants by the effluents released from a paper mill near Nanjangud lead to the accumulation of heavy metals in soil and different parts of paddy crop. The concentration of heavy metals except Zn in the seed was remarkably less than in the roots and leaves. The heavy metal uptake by plants showed greatest accumulation of Cu, Cr, Co and Pb in roots, Cd and Ni in leaves and Zn in seeds. Hayat *et al.* (2000) at Aligarh applied treated wastewater from Mathura oil refinery and ground water to mustard. Treated water had higher quantities of nutrients and heavy metals as compared to ground water. Accumulation of certain potentially toxic heavy metals was also recorded in seeds, but their concentration in soil and biomass was within the permissible limits. Further the heavy metal content of soil was not significantly co-related with that of seeds. El-Motaium and Badawy (2000) at Cairo (Egypt) collected soil (rhizosphere and bulk soils) and plants, cabbage and orange tree sample to evaluate the effect of sewage wastewater. An increase in organic matter and clay content and electrical conductivity of soil was observed. Continuous reduction in $CaCO_3$ and pH values of both the soils was developed. Total HNO_3 extractable and DTPA extractable heavy metals Fe, Mn, Cu, Zn, Cd, Co, Ni and Pb in both rhizosphere and bulk soils of cabbage and orange trees increased as irrigation period increased. Heavy metal concentration in cabbage roots was lower than their concentration in orange roots under the same irrigation period. Heavy metal content in cabbage plant was in the order, root>leaves>stem whereas for orange trees the order was roots>leaves>fruit peel>fruit pulp. Accumulation was higher in orange tree roots than cabbage roots, but their accumulation in cabbage leaves was higher than in orange leaves.

In Mexico, Amodo Alvarez and Franco (2001) irrigated Oats with waste water from a lake. Fertilizers like nitrogen, phosphorus and potassium were also used at varying levels i.e. 0, 80, 160, 240 kg N ha^{-1} and 0, 40, 80, and 120kg P ha^{-1} and 100kg K ha^{-1} along with a control. Soil, irrigation water and oats plants were analysed for heavy metals. No heavy metals were detected in oats, although irrigation water and soil was contaminated with Pb, Cd, Ni, Co and Cr. As a result of direct use of sewage water for a very long period of time to irrigate crops heavy metals have accumulated in these soils. Vazquez-Alarcon *et al.* (2001) performed studies in Hidalgo state, (Mexico) for the diagnosis of accumulation and variability of the concentration of Cd, Ni and Pb in water, soil as well as in maize, wheat and leucerne. Water sample, soil sample and samples of foliar tissues and grains of maize and wheat were taken from seven sites with a variation in the time of use of wastewater. Total Pb concentration in the water of gran canal del desague was 0.13 mg l^{-1}, while in the water of dam was 0.054 mg l^{-1}. The division of concentration of Cd in foliar tissue and in the grain of wheat decreased in four of seven sites. At Tlahualilpani site the variation was 1.5 (March) to 0.9 (April), this showed that Cd was transferred to grain and from there to food chain. Lead stayed in wheat leaves among the five of the seven sites, the calculated co-efficient increased from 0.8 (March) to 2.4 (April) in the same site.Ni coefficient in wheat decreased at seven sites and this showed that Ni was transferred to the grain.

Awan *et al.* (2002) at Faisalabad (Pakistan) while studying the suitability of industrial wastewater and saline water to sisoo seedlings used three treatments. T1 control (EC 2 dsm^{-1}) 50% solution, T2 (EC 10 dsm^{-1}) with salts and industrial effluents + salts, T3 (EC 20 dsm^{-1}) salts only and salts and industrial effluents, reported that height at T3 was nearly half of the height obtained in T1. The combined effect of salts and industrial effluents significantly reduced the height due to Cu, Mn and Zn. Combination of industrial effluents and salinity reduced the leaf area more than that of single salt. Erfani *et al.* (2002) while working at Mashhad (Iran) applied five treatments of municipal wastewater T1 (irrigation with treated wastewater overall growing season), T2 (Alternate irrigation with treated wastewater and well water) and T3 (irrigation with well water and application of cattle manure), T4 (irrigation with well water plus fertilizer N and P), T5 (irrigation with well water only as control) to lettuce. Results revealed that yield was higher in T1, T2, T3 and T4 as compared to control and NPK and heavy metal concentration also increased. In T1, Fe was maximum and Cd minimum. It was also observed that EC, total nitrogen, available phosphorus, soluble boron and heavy metal concentration of soil irrigated with wastewater also increased.

At Aligarh, effect of wastewater on the heavy metal content of the soil and seeds of mustard was observed by Inam *et al.* (2002). They reported that heavy metals in soil, water and seeds were within permissible limits, further Ahmad *et al.* (2003) observed the response of sugarcane and reported that plants responded better to treated wastewater of refinery as compared to ground water. Soil receiving wastewater accumulated some heavy metals, whereas the plant samples receiving this water only exhibited presence of Ni, Pb and Zn which were within permissible limits.

MATERIALS AND METHODS

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MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

In order to assess the suitability of city wastewater (WW) for the purpose of irrigation and as a source of nutrients along with NPK applied as fertilizers, four pot experiments were conducted during the rabi seasons of 2002-2005. The crop mustard (*Brassica juncea* var. Varuna) was grown with two concentrations of wastewater i.e. 100%WW (raw waste water) and 50%WW (raw wastewater and ground water mixed in 1:1 ratio) in addition to ground water (GW) as control. It may be pointed out here that city wastewater is the mixed water of industrial, sewage and household wastewater which is thrown out in a drain going outside the city at Aligarh-Mathura road and is commonly used as a source of irrigation for various types of crops (Fig. 1).

3.1 Agroclimatic Conditions of Aligarh.

Aligarh city is situated in Western Uttar Pradesh, 135 Km away from Delhi, the capital of India. It occupies on area of 5024 Km^2 and is located at 27° 52' latitude and 78°51' longitude and has an elevation of 187.45m. The climate is semi-arid and subtropical with severe hot dry summers and intense cold winters. Winter starts from October and ends in March, where December and January are the coldest months, as the average temperature ranges between 13°C to 15°C in these two months, while the minimum temperature may go down upto 2°C. Summer extends from April to June and the temperature goes upto 46 or 47 °C in the months of May and June (Fig. 2). Maximum rainfall occurs from July to September and records an average of about 850 mm and about 10% rainfall commonly occurs in winter which is useful for rabi crops. Soils found in Aligarh district are sandy, clayey, sandy loam and clayey loam. However the soil used for the present study was sandy loam.

3.2 Pot Preparation and Seed Treatment

For starting each experiment, earthen pots of 12" diameter were filled with soil at the rate of 7kg each pot. Soil was mixed thoroughly with farmyard manure in the ratio of 3:1 so as to maintain the organic matter. Inorganic fertilizers were added one day prior to sowing to avoid seed injury. Fertilizers were calculated on the basis that

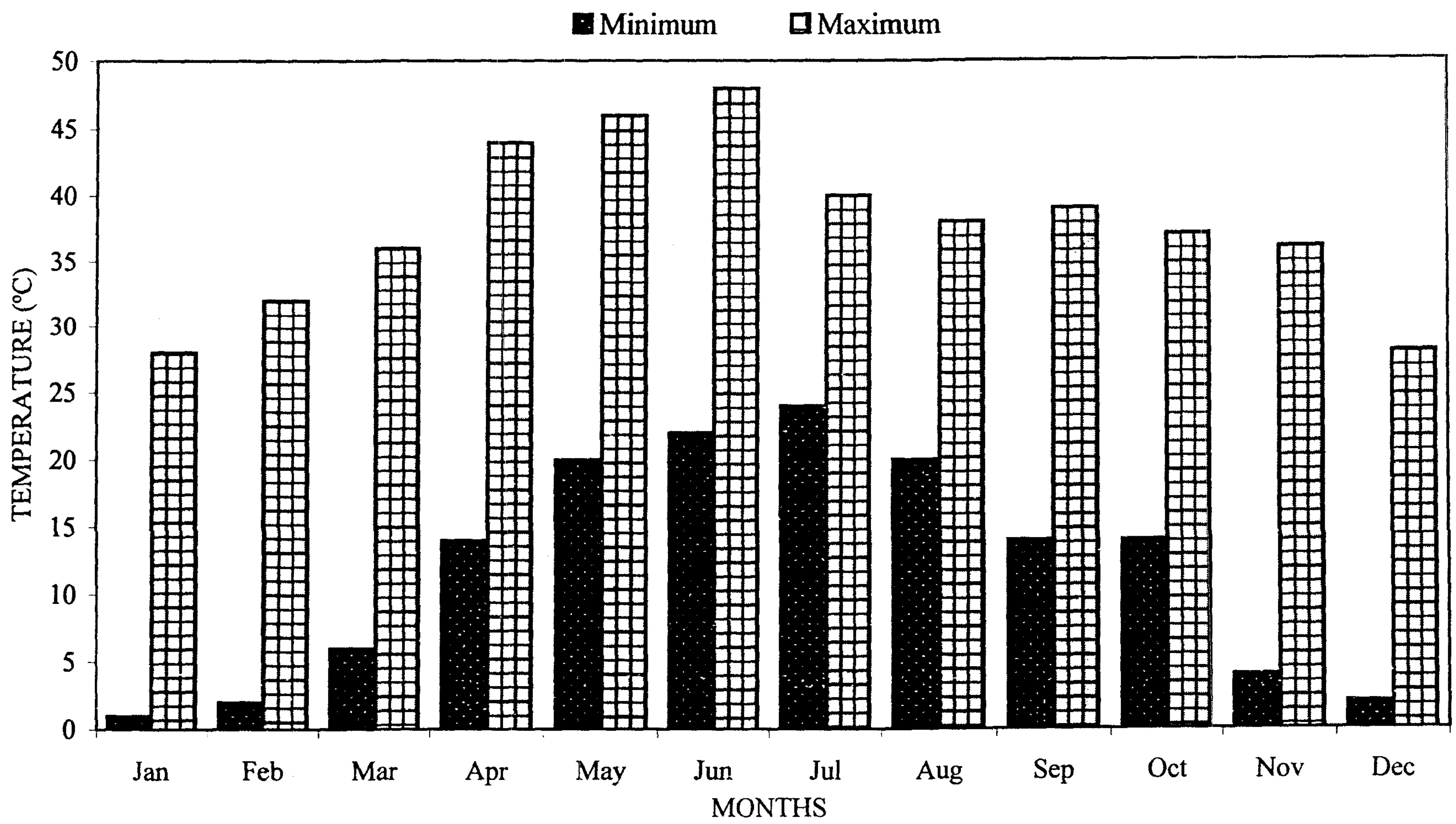

Fig. 2. Monthly temperature variation at Aligarh

one hectare land contains 2×10^6 kg effective soil (Singh, 1988). The sources of fertilizers were urea for nitrogen, single superphosphate for phosphorus, and muriate of potash for potassium. Before sowing, light application of GW was given to the pots so as to provide the moisture necessary for germination. Authentic seeds of *Brassica Juncea* var. Varuna obtained from Indian Agricultural Research Institute (IARI) New Delhi were surface sterilized with absolute alcohol and dried in shade. The seeds were sown at the rate of 10 per pot so as to avoid germination failure. After the establishment of seedlings i.e. 12 days after sowing, thinning was done, retaining only one healthy looking plant in each pot of more or less uniform size in 15 pots for each treatment. Three pots were randomly selected for three replicates at each sampling and in all three samplings were undertaken at vegetative, flowering and fruiting stages. Remaining six pots were used at the harvest for the study of yield and its parameters. Pots were watered on alternate days because of winters with 500 ml of water uniformly upto the maturity of crop. Weeding was done whenever necessary.

3.3 Experimental Scheme

Experiments were conducted according to complete randomized block design.

3.3.1 Experiments I

First two experiments were conducted simultaneously during the rabi season of 2002-2003. The aim of first experiment was to study the effect of two levels of WW i.e. 100% WW, 50% WW and GW, with four levels of nitrogen i.e. N_0, N_{40}, N_{80} and N_{120} (Table1) on the performance of one variety of mustard Varuna. Uniform dose of 20kg P and 20 kg K ha^{-1} was also applied basally to maintain the fertility of soil.

3.3.2 Experiment II

In experiment II water treatments were the same, but with four different levels of phosphorus i.e. P_0, P_{15}, P_{30} and P_{45}. Here also a uniform basal dose of 40 kg N ha^{-1} and 20 kg k ha^{-1} was applied one day before sowing (Table 1). Seeds for the Experiment I were sown on 18th of November and for Experiment II on 19th of November 2002 and the crop was harvested on 20th and 21st March 2003 respectively. During the harvesting the plants were uprooted carefully and dried in shade.

Table 1. Scheme of treatments for Experiments I and II.

Experiment -I

N Treatments (kg N ha^{-1})	Irrigation water treatments			Remarks (mg N kg^{-1} soil)*
	GW	50%WW	100%WW	
N_0	+	+	+	No fertilizer
N_{40}	+	+	+	20
N_{80}	+	+	+	40
N_{120}	+	+	+	60

A uniform basal dose (20 kg each of P and K ha^{-1}) was applied one day before sowing.

Experiment -II

P Treatments (kg P ha^{-1})	Irrigation water treatments			Remarks (mg P kg^{-1} soil)*
	GW	50%WW	100%WW	
P_0	+	+	+	No fertilizer
P_{15}	+	+	+	7.5
P_{30}	+	+	+	15.0
P_{45}	+	+	+	22.5

A uniform basal dose (40 kg N and 20 kg K ha^{-1}) was applied one day before sowing.

* calculated on the basis that one hectare land contains 2×10^6 kg effective soil.

3.3.3 Experiment III

This experiment was conducted during the rabi season of 2003-2004 under the same three levels of water GW, 50% WW and 100% WW on the same crop and variety, grown under four different levels of potassium i.e. K_0, K_{10}, K_{20} and K_{30} (Table 2). Crop was supplied with uniform basal dose of 80 kg N and 30 kg ha^{-1}. Sowing was done on 15th November 2003 and harvesting on 16th March 2004.

3.3.4 Experiment IV

Since individual optimum doses of nitrogen, phosphorus and potassium in presence of wastewater irrigation were worked out in the earlier three experiments, therefore Experiment IV was conducted to test the effect of optimum doses obtained in experiments I-III together. In this pot trial optimum dose however, was split so as to observe if some fertilizer can further be saved (Table 2). The crop was sown on 20th November 2004 and harvested on 19th March 2005.

3.4 Statistical Analysis of the Collected Data

The data obtained were analysed statistically taking into consideration the variables in each experiment according to Panse and Sukhatme (1985). The 'F' test was applied to assess the significance of data at 5% level of probability ($P \leq 0.05$). The error due to replication was also determined. The model of analysis of variance (ANOVA) is given. Critical difference (CD) was calculated to compare the mean values of various treatments (Table 3). Correlation coefficient values (r) were also obtained with some growth and yield parameters and seed yield.

3.5 Soil Analysis

Before sowing soil samples were taken in small quantity randomly from each pot grounded with the help of mortar and pestle and passed through a 2 mm sieve, then it was analysed for the following physico-chemical characteristics (Table 4).

3.5.1 Soil Texture

It was determined by the field method by rubbing or feeling the soil between thumb and fingers. The soil was moderately gritty, formed fairly firm ball which was easily broken and stained the finger indicating the characteristics of sandy loam soil.

Table 2. Scheme of treatments for Experiments III and IV.

Experiment -III

K Treatments (Kg K ha^{-1})	Irrigation water treatments			Remarks (mg K kg^{-1} soil)*
	GW	50%WW	100%WW	
K_0	+	+	+	No fertilizer
K_{10}	+	+	+	5
K_{20}	+	+	+	10
K_{30}	+	+	+	15

A uniform basal dose (80 kg N and 30 kg P ha^{-1}) was applied one day before sowing.

Experiment -IV

Treatments (Kg NPK ha^{-1})	Irrigation water treatments			Remarks (mg NPK kg^{-1} soil)
	GW	50%WW	100%WW	
$N_0P_0K_0$ (Control)	+	+	+	No fertilizer
$N_{40}P_{15}K_{10}$ (Optimum)	+	+	+	(20+7.5+5)
$N_{80}P_{30}K_{20}$ (Full)	+	+	+	(40+15+10)

Starter doses in experiments III and IV were the optimum doses obtained in experiments I and II.

* calculated on the basis that one hectare land contains 2×10^6 kg effective soil.

Table 3. Model of analysis of variance (ANOVA) of Experiment I (Experimental design; randomized complete block design)

Source of variation	df	SS	MSS	F. value	Sig.
Replication	2				
Water/wastewater	2				
Nitrogen (N)	3				
Interaction	6				
Error	22				
Total	35				

Table 3. Model of analysis of variance (ANOVA) of Experiment II (Experimental design; randomized complete block design)

Source of variation	df	SS	MSS	F. value	Sig.
Replication	2				
Water/wastewater	2				
Phosphorus (P)	3				
Interaction	6				
Error	22				
Total	35				

Table 3. Model of analysis of variance (ANOVA) of Experiment III (Experimental design; randomized complete block design)

Source of variation	df	SS	MSS	F. value	Sig.
Replication	2				
Water/wastewater	2				
Potassium (K)	3				
Interaction	6				
Error	22				
Total	35				

Table 3. Model of analysis of variance (ANOVA) of Experiment IV (Experimental design; randomized complete block design)

Source of variation	df	SS	MSS	F. value	Sig.
Replication	2				
Water/wastewater	2				
Fertilizer (NPK)	2				
Interaction	4				
Error	16				
Total	26				

3.5.2 Cation Exchange Capacity (CEC)

CEC of soil was determined by the method of Ganguly (1951). To 10 g soil, 0.2N HCl was added. It was shaken for 30 minutes, filtered and washed with DDW, till it became free from chloride ions, which was checked with $AgNO_3$. The residue was transferred from the filter paper to a beaker and suspension of known concentration was prepared. It was then treated with 10 ml of standard KCl solution, shaken for 30 minutes and left overnight. Then it was titrated with 0.1N NaOH, using phenolphthalein indicator (Appendix, iii). From the amount of NaOH required, the CEC of samples was calculated as follows.

$$\text{CEC (meq 100g}^{-1}) = \frac{\text{Volume of 0.1N NaOH} \times \text{N of NaOH}}{\text{Weight of the Sample}}$$

3.5.3 Hydrogen ion Concentration (pH)

It was estimated with the help of pH meter. To 10 g soil, 50 ml of double distilled water (DDW) was added and shaken thoroughly. After 30 minutes, pH of the suspension was recorded. The pH meter was calibrated with a standard buffer of known pH (Jackson, 1973).

3.5.4 Organic Carbon

It was estimated by the method of Walkley and Black (1934). 2 g sample was taken in a 500 ml conical flask. To this 10 ml of 1N potassium dichromate solution (Appendix, iv) and 20 ml concentrated sulphuric acid were added. After shaking for about 2 minutes, it was kept on an asbestos mat for 30 minutes. 200 ml DDW, 10 ml phosphoric acid (85%) and 1 ml of diphenyl amine indicator (Appendix, ii) were added. Deep violet colour appeared which was titrated with 0.5N ferrous ammonium sulphate solution (Appendix, ii) till the colour changed to purple and finally green. Simultaneously a blank was run without sample.

$$\text{\% of Organic Carbon} = \frac{\text{Blank titre - Actual titre}}{\text{Weight of Sample}} \times 0.003 \times 100 \times \text{N}$$

where N = normality of ferrous ammonium sulphate

3.5.5 Nitrate Nitrogen

It was estimated according to the method of Ghosh *et al.* (1983). 20g soil were shaken continuously with 50 ml DDW for 1 hour in a 100 ml conical flask fitted with rubber stopper. A pinch of $CaSO_4$ was added and shaken. Then the contents were filtered through a Whatman fitter paper No 1. 20 ml clear filterate was transferred to 50 ml porcelain dish and was evaporated to dryness on steam bath. After cooling 3 ml phenol disulphonic acid (Appendix, iii) was added and allowed to react for 10 minutes. 15 ml DDW was added and stirred with glass rod until the residue was dissolved. After cooling the contents were washed down into 100 ml volumetric flask, to this 1:1 liquid ammonia (Appendix, ii) was added slowly and mixed well, till the solution became alkaline which was indicated by the appearance of yellow colour due to presence of nitrate. Then another 2 ml ammonia was added and finally the volume was made upto 100 ml with DDW. The intensity of yellow colour was read at 410 nm with spectrophotometer.

For standard curve, stock solution containing 100 ppm nitrate was prepared by dissolving 0.722g potassium nitrate in DDW and the volume was made upto 1 litre. This was diluted 10 times to give 10 ppm NO_3^--N solution. Aliquots (2, 5, 10, 15, 20 and 25 ml) were evaporated on water bath to dryness in small porcelain dishes. After cooling, 3 ml phenol disulphonic acid was added and yellow colour was read as described above, simultaneously a blank was also run.

3.5.6 Phosphorus

To 2.5 g soil sample in 100 ml conical flask, a pinch of Draco G60 was added followed by 50 ml of Olsen's reagent (Appendix, iii). The flask was shaken for about 30 minutes on a shaker and then the contents were filtered through a Whatman filter paper No 1. In the filterate phosphorus was estimated through spectrophotometer by Dickman and Bray's (1940) method.

5 ml soil extract was pipetted into a 25 ml volumetric flask and 5 ml Dickman and Bray's reagent (Appendix, i) was poured drop by drop with constant shaking till effervescence due to CO_2 evolution ceased. The inner wall of the flask neck was washed with DDW and the contents diluted to about 22 ml. Then 1 ml stannous chloride solution (Appendix, iv) was added and the volume was made upto the mark. The intensity of blue colour was read at 660 nm on spectrophotometer. For the standard curve 0.439 g potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in half litre of DDW. To this 25 ml 7 N sulphuric acid (Appendix, v) was added and volume was maintained upto 1 litre with DDW, giving 100 ppm stock

Table 4. Physico-chemical characteristics of soil tested before sowing. All determinations in $mg l^{-1}$ in 1:5 (soil-water extract) or as specified.

Determinations	Experiments			
	I	II	III	IV
Texture	Sandy Loam			
CEC (meq $100 g^{-1}$ soil)	3.42	2.93	3.26	3.18
pH	7.60	7.50	8.00	7.80
Organic carbon (%)	0.850	0.798	0.634	0.809
EC (μ mhos cm^{-1})	238.00	294.00	286.00	315.00
NO_3^-–N (g kg^{-1} soil)	0.322	0.287	0.310	0.289
Phosphorus (g kg^{-1} soil)	0.115	0.118	0.126	0.121
Potassium	16.00	15.00	17.00	18.00
Calcium	30.90	29.60	31.12	32.16
Magnesium	18.81	17.86	19.05	18.26
Sodium	13.24	14.92	14.06	14.13
Carbonate	18.64	19.48	20.06	19.84
Bicarbonate	109.76	98.62	110.32	90.06
Sulphate	16.32	14.98	18.46	15.01
Chloride	30.99	28.19	26.56	24.32

solution of phosphorus. From this, 2 ppm solution was made after 50 times dilution. For the preparation of standard curve different concentrations of P (1, 2, 3, 4, 5 and 10 ml of 2 ppm phosphorus solution) were taken in 25 ml volumetric flasks. To these, 5 ml of extracting reagent (Olsen's reagent) was added. The colour was developed by adding Dickman and Bray's reagent and stannous chloride and read at 660nm. A blank was run without the sample. The curve was plotted and the amount of P was calculated from the curve.

3.5.7 Potassium

5g soil was shaken with 25 ml 1N ammonium acetate (Appendix, i) for 5 minutes and was filtered immediately through a Whatman filter paper No 1. Stock solution of 1000 ppm K was prepared by dissolving 1.908g KCl in 1 litre DDW. From the stock solution aliquots were diluted in 50 ml volumetric flask with ammonium acetate solution to give 10 to 40 ppm of K. These were read with the help of flame photometer after setting zero for the blank at 100 for 40 ppm of K. The curve was obtained by plotting the readings against the different concentrations (10, 15, 20, 25, 30, 35, and 40 ppm) of K.

3.5.8 Preparation of Soil Extract for Calcium, Magnesium, Chloride Carbonate, Bicarbonate and Sulphate

100 g sample transferred to 750 ml flask, to which 500 ml DDW was added and the flask was shaken for about 1 hour. The contents were filtered through Buchner funnel.

3.5.8.1 Calcium

It was estimated according to the method of Chopra and Kanwar (1982). To 25 ml extract, 2-3 crystals of carbamate and 5 ml of 16% NaOH solution were added. Then it was titrated against 0.01M EDTA (Appendix, ii) using murexide indicator (Appendix, iii) till the colour changed from orange red to purple.

3.5.8.2 Magnesium

To 25 ml sample extract, 5 ml ammonium chloride-ammonium hydroxide buffer (Appendix, i) was added followed by titration with 0.01M EDTA using Erichrome Black-T (Appendix, ii) as an indicator, the colour changed from green to wine red (Chopra and Kanwar, 1982).

3.5.8.3 Chloride

50 ml sample extract was taken in a flask and 2 ml of 5% K_2CrO_4 indicator (Appendix, iv) was added. It was titrated against 0.02N silver nitrate solution (Appendix, iv)

$$\text{Chloride mgl}^{-1} = \frac{(\text{ml} \times \text{N}) \text{of Ag NO}_3 \times 1000 \times 35.5}{\text{ml of sample}}$$

3.5.8.4 Carbonates and Bicarbonates

Estimation was done following the method of Richards (1954). 50 ml soil sample extract was taken in a flask. To this, 5 drops of phenolphthalein indicator were added. The appearance of pink colour indicated the presence of carbonates. Then it was titrated against 0.01N sulphuric acid till the solution became colourless. To the colourless solution from the above titration, 2 drops of 0.05% methyl orange indicator (Appendix, ii) were added. It was then titrated against 0.01N sulphuric acid till the colour changed from yellow to rose red. This indicated the presence of bicarbonates in the sample.

$$\text{(a) carbonates (meq l}^{-1}) = 2\text{Y} \times \text{N of H}_2\text{SO}_4 \times \frac{1000}{\text{ml aliquot}}$$

$$= 2\text{Y} \times 2$$

$$\text{(b) bicarbonates (meq l}^{-1}) = (\text{Z} - 2\text{Y}) \times \text{N of H}_2\text{SO}_4 \times \frac{1000}{\text{ml aliquot}}$$

$$= (\text{Z} - 2\text{Y}) \times 2$$

where Y= reading of burette for the titration of carbonate

Z = reading of burette for the titration of bicarbonate

3.5.8.5 Sulphate

To 50 ml sample extract, 2.5 ml conditioning reagent (Appendix, i) was added. It was then stirred on a shaker and during shaking small quantity of barium chloride was added. It was then read with the help of nephlometer.

$$\text{SO}_4 \text{ (mg l}^{-1}) = \frac{\text{mg SO}_4 \times 1000}{\text{ml sample}}$$

3.5.9 Sodium

Determination of Na was carried out directly from the soil extract (1:5, soil : water) with the help of flame photometer. Standard curve was prepared by taking

5.845g NaCl dissolved in DDW and was made upto 1 litre that gave 100 milli equivalents litre^{-1} of Na. From this stock solution, dilutions containing 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 meq Na litre^{-1} were prepared. Plotting the flame photometer readings on Y-axis against concentrations of Na on X-axis, a curve was drawn. Na in the unknown sample was read from the curve.

3.6 Water Sampling and Analysis

Wastewater was collected in sample bottles for analysis twice and in 50 litre jerry canes for watering to pots as and when required. For water analysis, sample bottles were carefully cleaned before use with chromic acid clearing mixture. Then the bottles were rinsed thoroughly with tap water and then with distilled water. 4 litres of wastewater sample was taken for analysis. Prior to filling, the bottles were again rinsed with city wastewater to be collected. Tap water was used as GW. In order to obtain 50% WW both the waters i.e. WW and GW were mixed in 1:1 ratio. All the three waters were analysed for following characteristics (Table 5).

3.6.1 Colour

Colour in water may be due to the presence of fine particles in suspension or due to mineral matter in solution and it was light black.

3.6.2 Odour

It results because of the micro organisms and certain gases generally present in wastewater and it was slightly unpleasant.

3.6.3 Hydrogen Ion Concentration (pH)

It was determined with the help of pH meter. The pH meter was adjusted before use with standard buffer of known pH.

3.6.4 Electrical Conductivity (EC)

It was directly read with the help of conductivity meter by putting the sample in a beaker. The apparatus was adjusted to 25°C of the solution.

3.6.5 Total Dissolved Solids (TDS)

100 ml filtered sample was taken in an evaporating dish and allowed to evaporate on water bath.

$$\mathrm{TDS}\,(\mathrm{gl}^{-1}) = \frac{\mathrm{A} - \mathrm{B} \times 1000}{\mathrm{V}}$$

Table 5. Physico-chemical characteristics of ground water (GW) and 50% city wastewater (50%WW) and 100% city wastewater (100%WW). All determinations in mg l^{-1} or as specified.

	2002-2003 (Experiment I & II)						2003-2004 (Experiment III)						2004-2005 (Experiment IV)						
	Sampling I			Sampling II			Sampling I			Sampling II			Sampling I			Sampling II			
	GW	50%	100%	GW	50%	100%	GW	50%	100%	GW	50%	100%	GW	50%	100%	GW	50%	100%	
Colour								Light Black											
Odour								Slightly Unpleasant											
pH*	7.8	7.0	6.9	8.1	7.3	7.0	7.6	7.3	7.1	7.8	7.3	6.9	7.8	7.3	7.0	8.0	7.6	7.0	FAO
EC (µ mohs cm^{-1})*	710	1040	1380	730	1070	1400	750	1060	1370	790	1100	1400	760	1080	1380	760	1080	1370	FAO
TDS*	540	858	1410	528	741	1296	526	903	1540	554	890	1422	553	936	1358	503	921	1379	FAO
TSS	448	593	1070	442	496	734	408	499	826	405	506	832	443	669	942	448	672	981	
TS	988	1451	2480	970	1237	2030	934	1402	2366	959	1396	2254	996	1605	2300	951	1593	2360	
BOD**	17.34	74.41	140.32	16.16	70.51	128.14	17.06	78.42	128.12	16.54	71.28	133.11	17.63	68.41	151.28	17.18	78.30	121.43	CPCB 1995
COD	42.32	210.18	363.24	38.22	213.51	368.17	45.26	200.30	349.42	41.32	213.16	351.20	39.12	215.45	370.36	42.60	217.13	371.18	
Ca*	28.12	99.91	152.66	26.12	91.55	155.41	28.96	91.03	152.04	25.45	89.95	155.66	27.54	90.16	151.96	27.01	90.16	152.85	FAO
Mg**	18.32	72.75	128.62	17.54	73.53	129.13	17.33	73.71	128.41	17.12	79.14	129.54	18.06	78.58	128.13	17.14	80.15	128.77	FAO
K	8.39	13.24	19.91	7.82	13.13	19.58	8.06	13.78	20.03	8.01	13.54	19.64	8.45	14.06	19.92	8.21	13.75	19.81	
Na*	15.60	25.81	40.12	15.24	26.32	43.26	14.80	29.65	42.41	15.73	21.95	38.26	15.92	30.23	46.62	15.02	24.60	42.24	FAO
HCO_3^- *	64.16	220.16	374.12	66.12	218.17	366.44	61.23	218.38	379.53	71.25	214.14	356.23	65.34	212.13	349.52	58.64	210.91	351.21	FAO
CO_3^-	18.16	57.76	96.14	21.82	58.12	92.41	18.56	59.80	90.32	19.91	61.54	101.57	17.96	58.56	98.19	18.23	50.16	101.34	
Cl^- *	71.42	101.18	136.17	65.37	96.18	132.18	68.51	102.18	144.21	66.14	91.70	131.26	75.23	92.57	130.24	71.26	91.93	124.61	FAO
PO_4	0.76	1.31	1.90	0.73	1.12	1.61	0.68	1.03	1.84	0.66	1.24	1.81	0.71	1.17	1.76	0.74	1.03	1.70	
NO_3-N*	0.80	1.06	1.24	0.74	0.99	1.17	0.76	1.02	1.28	0.71	0.89	1.12	0.86	1.04	1.19	0.88	0.98	1.03	FAO
NH_3-N*	2.36	3.91	5.84	2.02	3.80	5.91	2.01	3.64	5.70	2.12	3.89	5.64	2.18	3.50	5.54	2.05	3.50	5.40	FAO
SO_4*	41.26	55.17	70.13	39.16	51.06	75.08	38.14	57.21	82.33	45.61	55.12	80.14	44.18	52.31	76.15	41.19	56.21	76.41	FAO

* values were within the limits of FAO 1994 or Central Pollution Control Board 1995 for irrigation water.

** values crossed the limits of FAO 1994 or Central Pollution Control Board 1995 for irrigation water.

Table 6a. Heavy metal contents of wastewater (All determinations in mg l^{-1}).

	Experiments I & II	Experiment III	Experiment IV
Cadmium (Cd)	0.006	0.005	0.006
Chromium (Cr)	0.044	0.043	0.041
Nickel (Ni)	0.491	0.496	0.480
Lead (Pb)	0.031	0.035	0.029

Table 6B. Heavy metal contents in soil (All determinations in µg g^{-1}).

	Experiments I & II	Experiment III	Experiment IV
Cadmium (Cd)	0.23	0.27	0.24
Chromium (Cr)	0.053	0.051	0.056
Nickel (Ni)	0.81	0.79	0.83
Lead (Pb)	0.75	0.78	0.79

Where A = Final weight of dish

B = Initial weight of dish

V = Volume of the sample taken

3.6.6 Total Suspended Solid (TSS)

These were determined by calculating the difference between the total solids and total dissolved solids.

$$TSS\ (gl^{-1}) = TS - TDS$$

3.6.7 Total Solids (TS)

100 ml unfiltered sample was taken on evaporating dish and was allowed to evaporate on water bath.

$$\text{Total Solids}\ (gl^{-1}) = \frac{A - B \times 1000}{V}$$

Where A = Final wt of dish

B = Initial wt of dish

V = Volume of the sample taken

3.6.8 Dissolved Oxygen (DO) & Biological Oxygen Demand (BOD)

Different volumes of the samples were placed in BOD bottles (250 ml) to get several dilutions of the samples to obtain the required depletions ranging between 0.1 and 1.0%. These bottles were filled with DDW, stoppered and one set of bottles was incubated for 5 days in an incubator maintained at 20°C and in other set, dissolved oxygen (DO) was determined immediately by adding 2 ml manganous sulphate solution (Appendix, iii) followed by 2 ml alkali iodide azide reagent (Appendix, i) by means of graduated pipette by dipping its end below the surface of the liquid. The BOD bottles were stoppered and mixed well by inverting. The bottles were then allowed to stand till the precipitate settled half way, leaving clear supernatant above the manganese hydroxide flakes. The stopper was removed and 2 ml H_2SO_4 was immediately added. Each bottle was re-stopered and the contents were mixed by gentle inversion until dissolution was complete. 200 ml sample was taken in 500 ml conical flask, and then 2 ml starch indicator (Appendix, iv) was added and titrated against 0.025N sodium thiosulphate solution (Appendix, iv) till the disappearance of blue colour. The reading of sodium thiosulphate used up was indicative of DO of the sample in mg l^{-1}. BOD was calculated using following relationship

$$BOD\ mg\ l^{-1} = \frac{D1 - D2}{P}$$

where D1 and D2 are the DO of the diluted samples, 15 minutes after the preparation of the sample and after 5 days of incubation respectively and P is the decimal fraction of the sample used.

3.6.9 Chemical Oxygen Demand (COD)

0.4g mercuric sulphate was placed in a refluxing flask and 20 ml sample was added. Both were mixed well and 10 ml 0.25N potassium dichromate solution was added followed by 30 ml sulphuric acid and small amount of silver sulphate. A blank was run using distilled water. These were subjected to reflux for two hours, cooled and then diluted to 100 ml DDW. The contents were then titrated againt 0.1N ferrous ammonium sulphate solution.

$$COD\ mg\ l^{-1} = \frac{A - B \times N \times 8000}{ml\ sample}$$

where A = ml ferrous ammonium sulphate used for blank titration.

B = ml ferrous ammonium sulphate used for sample titration

N = Normality of ferrous ammonium sulphate solution

3.6.10 Calcium

50 ml sample was taken in a conical flask and neutralized with acid. It was boiled for 1 minute and then cooled. 2 ml 1N sodium hydroxide solution (Appendix, iv) was added to maintain pH at 12-13. After the addition of 1-2 drops of ammonium purpurate indicator (Appendix, i), it was titrated slowly with 0.01M EDTA and calculated as follows.

$$Ca\ (mg\ l^{-1}) = \frac{A \times B \times 400.8}{ml\ sample}$$

where A = ml titration for sample.

B = mg $CaCO_3$ equivalent to 1.0 ml EDTA titrant at the calcium indicator end point.

3.6.11 Total Hardness

50 ml of water sample was taken in a conical flask and 1 ml ammonium chloride – ammonium hydroxide buffer solution was added. After the addition of 100 mg erichrome black-T indicator, it was titrated against 0.01M EDTA solution.

$$\text{Total hardness as mgl}^{-1}\ CaCO_3 = \frac{\text{ml of EDTA used} \times 1000}{\text{ml sample}}$$

3.6.12 Magnesium

It was estimated from EDTA and hardness titration (taken from total hardness estimation).

Mg (mg l^{-1}) = Total hardness (as mg $CaCO_3l^{-1}$) – Calcium hardness × 0.244 (as mg $CaCO_3l^{-1}$)

3.6.13 Potassium

The estimation of potassium was carried out directly with flame photometer at 768 nm using appropriate filter and a standard curve by taking known concentrations of potassium. A stock solution of 1000 ppm K was prepared by dissolving 1.908 g KCl in DDW. From the stock solution aliquots were diluted in 50 ml volumetric flask with ammonium acetate solution to give 10 to 40 ppm of K. These were read with the help of flame photometer after setting zero for blank at 100 for 40 ppm K. The curve was obtained by plotting the readings against the different concentrations (10, 15, 20, 25, 30, 35, and 40 ppm) of K.

3.6.14 Sodium

Sodium was also estimated flame photometrically at 589nm using specified filter and standard curve by taking known concentrations of sodium salt. For standard curve 5.845 g sodium chloride was dissolved in DDW and volume maintained at 1 litre. This gave 100 meq l^{-1} of Na. From this stock solution dilutions containing 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 meq Na l^{-1} were prepared. Plotting the flame photometer readings on Y-axis against concentrations of sodium on X-axis, a curve was drawn. The concentration of sodium in the unknown sample was read from the curve.

3.6.15 Carbonate and Bicarbonate

Estimation was done following the method of Richards (1954) 50 ml water sample was taken in a clear flask. To this 5 drops of phenolphthalein indicator were added. The appearance of pink colour indicated the presence of carbonate, then it was titrated against 0.01N sulphuric acid till the solution turned colourless. To the above solution, 2 drops of methyl orange indicator were added. It was again titrated against

0.01N H_2SO_4 till the colour changed from yellow to rose red. This indicated the bicarbonate presence.

$$\text{(a) carbonate (meq l}^{-1}) = 2Y \times \text{normality of } H_2SO_4 \times \frac{1000}{\text{ml aliquot}}$$

$$= 2Y \times 2$$

$$\text{(b) bicarbonate (meq l}^{-1}) = (Z - 2Y) \times \text{normality of } H_2SO_4 \times \frac{1000}{\text{ml aliquot}}$$

where Y = reading of burette for titration of carbonate

Z = reading of burette for titration of bicarbonate

3.6.16 Chloride

50 ml sample was taken in a flask and 2 ml potassium chromate indicator was added. It was titrated against 0.02N silver nitrate solution

$$\text{Chloride mg l}^{-1} = \frac{(\text{ml} \times \text{N of } AgNO_3) \times 1000 \times 35.5}{\text{ml sample}}$$

3.6.17 Phosphate

To 100 ml sample containing not more than 0.2 mg phosphorus and free from colour and turbidity 0.05 ml phenolpthalein indicator was added. Sample turned pink. Strong sulphuric acid solution (Appendix, v) was added drop wise to discharge the colour. Smaller sample was taken and diluted to 1000 ml with DDW. After discharge of the pink colour with acid, 4 ml of 2.5% ammonium molybedate reagent (Appendix, i) was added. After 10 minutes the colour was measured with the help of spectrophotometer at 690 nm and comparison with the calibration curve was made using DDW blank.

$$\text{P mg l}^{-1} = \frac{\text{mg P} \times 1000}{\text{ml sample}}$$

3.6.18 Nitrate Nitrogen

First nitrate standard was prepared in the range of 0.1 to 1.0 mg l^{-1} N by diluting 1, 2, 4, 7 and 10 ml standard nitrate solution to 10 ml with DDW. Residual chlorine in the sample was removed by adding 1 drop sodium arsenite solution for each 0.1 mg Cl and mixed. One drop was added in excess to 50 ml portion. For colour development, number of reaction tubes were set in wire rack. To each tube 10 ml sample was added. The rack was placed in cool water bath and 2 ml NaCl solution was added and mixed well. Then 10 ml H_2SO_4 was added and cooled. 0.5 ml sulphanilic acid solution (Appendix,iv) was added and the tubes swirled to mix and then placed in water bath at not less than 95°C. After 20 minutes, it was taken out and cooled in a water bath. Reading was taken against a reagent blank at 410 nm. Standard curve was prepared from the absorbance values of the standard run together with the samples and correlated by subtracting their, sample blank, values from their final absorbance values. The concentration of NO_3–N was read directly from the standard curve.

3.6.19 Ammonia Nitrogen

For the estimation of ammonia nitrogen first preliminary distillation was performed. 500 ml ammonia free water was added to 20 ml borate buffer and the pH was adjusted to 9.5 with 6N sodium hydroxide solution. A few glass beads were added and the mixture was used to steam out the distillation apparatus until the distillate showed no trace of ammonia. For ammonia nitrogen content of less than 100 µg l^{-1}, volume of 4 litre was used. Residual chlorine was removed in the sample by adding dechlorintaing agent, 25 ml borate buffer was added and the pH was adjusted to 9.5 with 6N NaOH, using pH meter. Distillation of sample was done. The steaming out flask was disconnected and the sample was immediately transferred to the distillation apparatus. It was distilled at the rate of 6 to 10 ml minute^{-1} with the help of delivery tube submerged. The distillate was collected in 500 ml flask, containing 50 ml boric acid solution. At least, 300 ml distillate was collected. It was diluted to 500 ml with ammonia free water. 100 ml sample was taken in 500 ml Kjeldhal flask with ammonia free distilled water and diluted to 250 ml. Again it was distilled as before with few pieces of paraffin added to the distillation flask and 100 ml distillate was collected. Ammonia in the distillate was titrated against standard 0.02N H_2SO_4 titrant until the indicator turned to pale lavender. A blank was run through all the steps of the procedure.

$$\text{Ammonia N (mg l}^{-1}) = \frac{\text{A - B} \times 280}{\text{ml of sample}}$$

Where A = ml H_2SO_4 titration for sample

B = ml H_2SO_4 titration for blank

Table 7. Microbiology of waste water

Bacteria	Method used	Medium used	Bacterial count
Total heterotrophic bacteria	Spread plate method	Nutrient agar	2.29×10^7 CFU 100 ml^{-1}
Coliforms	MPN method	MacConkey broth	2.3×10^3 100 ml^{-1}
Faecal coliform	MPN method	EC broth	9.9×10^2 100 mi^{-1}
Salmonella-Shigella sp.	Spread plate method	SS agar	1.0×10^2 100 ml^{-1}

MPN = Most probable number; CFU = Colony forming unit

3.6.20 Sulphate

In a conical flask 100 ml sample was taken and 5 ml conditioning reagent was mixed. The contents of the flask were stirred for one minute on magnetic stirrer and small amount of barium chloride was added during stirring. It was read at 420 nm with the help of spectrophotometer. Standard sulphate solution was prepared by dissolving 0.1479g sodium sulphate in DDW, making the volume 1 litre. From this 0-40 mg l^{-1} dilutions were prepared at the interval of 5 mg l^{-1}. A standard curve was prepared by plotting the reading for each duration using spectrophotometer.

3.7 Microbiological Examination of Wastewater

The standard of wastewater used for crop irrigation was assessed (Table 7) as per the quality guideline of WHO (1989) and FAO (1994). The parameters like total bacterial viable count, coliforms, faecal coliforms and presence of Shigella and Salmonella were analysed using standard most probable number (MPN) and/or plate count methods as described by APHA (1998) and Cappucino and Sherman (1992).

Most Probable Number (MPN)

It is used to determine the mean concentration of coliform bacteria present in wastewater. For its estimation, samples were collected in sterile bottles, and diluted to 100 fold or as required in NSS. Then 3 series of Mac Conkey's broth tubes were made, first row with 5 tubes containing 10 ml double strength Mac Conkey's broth, second and third row with 5 tubes each containing single strength broth. Each tube should have one Durham's tube. Now 3 series were made for EC broth each row containing 5 tubes, each containing 5 ml of EC broth. Now 10 ml of diluted sample was inoculated in the first two tubes, 1 ml in second row tubes and 0.1 ml in third row tubes of Mac Conkey's broth. All the tubes were then incubated for 48 hrs at 37°C, then Mac Conkey's tubes were observed for gas production and total number of tubes for production was counted, then EC broth tube with 0.1 ml from positive tube of MacConkey was inoculated showing gas production. Then these tubes were incubated at 44.2°C in shaker incubation for 24 hours and the number of tubes for gas production was counted.

Plate Count Method

It is used to estimate the number of heterotrophic bacteria in water. Single colony is supposed to arise from single cell. However, it may arise from more than one cell. Therefore, instead of cell number, the term "Colony Forming Units" (CFU) are used to indicate bacterial viable count. 10 ml wastewater was mixed with 90ml sterie normal saline solution. From this 1ml was taken and serially diluted in 9ml of sterie NSS. 0.1ml sample was taken from the serially diluted tubes and spread on the above mentioned media, these plates were the incubated for 24-48 hours at 37°C (Cappucino and Sherman, 1992).

The colonies were counted as

CFU (colony forming unit)/ml

$$\text{CFU ml}^{-1} \text{ of water} = \frac{\text{Number of Colonies}}{\text{Dilution factor}}$$

Mean value of at least three samples indicated the most probable number of coliform 2.3×10^3 100 ml^{-1} of wastewater. Similarly, faecal coliform level was 9.9×10^2 100ml^{-1}. The above level of bacterial contamination is lower with the microbiology quality guideline of WHO (1989). Presence of Salmonella and Shigella like organisms on specific medium indicated the presence and survival of potentially pathogenic bacteria. Although no guideline is available for such bacteria for irrigation purpose.

Thus on the basis of microbiological quality assessment of wastewater and considering the present guideline of WHO it may be concluded that:

1. The wastewater requires treatment to meet the quality guideline to be used for crops to be eaten uncooked.
2. The wastewater may be recommended to be used for irrigating crops like cereal crops, fodder crops, pasture crops and trees.
3. Further investigation for the presence of protozoan cysts, viruses etc. may be helpful. Monitoring of such wastewater should be mandatory.

3.8 Biometric Observations

For investigating the comparative effect of WW, GW and fertilizers, observations were carried out at vegetative, flowering and fruiting stages of the crop.

3.9 Growth Characteristics

The following growth characteristics were observed at three stages

1. Plant height (cm)
2. Leaf number plant^{-1}
3. Leaf area (cm^2 plant^{-1})
4. Plant fresh weight (g plant^{-1})
5. Plant dry weight (g plant^{-1})

For assessing dry weight three plants from each treatment were dried, after taking their fresh weight, in hot air oven at 80°C for two days and weighed. Leaf area was measured by using leaf area meter (LA 211, Systronics, India).

3.10 Physiological Parameters

Following parameters were studied at three stages of growth.

Leaf Nitrate Reductase Activity (NRA)

It was estimated by the method of Jaworski (1971). Random samples of leaves from each plant were taken and cut into small pieces. 200 mg fresh leaf pieces were weighed and placed in polythene vials. To each 2.5 ml phosphate buffer (0.1M) pH 7.5 (Appendix, iii) and 0.5 ml potassium nitrate 0.2M solution (Appendix, iv) was added followed by addition of 2.5 ml 5% isopropanol (Appendix, ii). Lastly two drops of chloramphenicol solution were added to avoid bacterial growth in the medium. The vials were incubated for 2 hours in the dark at 30°C. 0.4 ml incubated mixture was taken in a test tube to which 0.3 ml of 1% sulphanilamide (Appendix, v) and 0.02% N-1 naphthylethylene diamine dihydrochloride (Appendix, iii) were added. The solution was left for 20 minutes for maximum colour development. It was diluted to 5 ml with DDW and optical density was read at 540 nm using spectrophotometer. A blank consisting of 4.0 ml DDW and 0.3 ml each of sulphanilamide and NED-HCl, was used simultaneously for comparison. Standard curve was plotted by taking known graded dilutions of sodium nitrate form a standard aqueous solution of this salt. The optical density of the sample was compared with the calibrated curve and NRA was expressed as μ mol $NO_2 g^{-1} h^{-1}$ fresh leaf tissue.

Carbonic Anhydrase (CA) Activity

Carbonic anhydrase was estimated by adopting the method of Dwivedi and Randhava (1974). The fresh leaf samples were cut into the small pieces at a temprature below 25°C. 200 mg of these leaf pieces were weighed and cut further into smaller pieces in 10 ml of 0.2 M cystein hydrocloride (Appendix, i) and left for 20 minutes at 4°C. The leaf pieces were taken out of the petriplates and adhering solution was soaked with the help of blotting paper. These desired samples were then transferred to a test tube containing 4 ml phosphate buffer of pH 6.8 (Appendix, iii). To this test tube 4 ml of 0.2 M sodium biocarbonate in 0.2N NaOH and 0.2 ml of bromothymol blue (0.002%) indicator (Appendix, i) were added. Test tubes were shaken gently and left for 20 minutes at 4°C. CO_2 liberated by the catalytic action of carbonic anhydrase on $NaHCO_3$ was estimated by titrating the reacting mixture against 0.01N hydrochloric acid using methyl red as an indicator. The control reaction mixture was also titrated against 0.01N HCl. In each case the quantity of HCl used to neutralize was noted and difference was calculated. The activity of enzyme was calculated by putting the values in the formula.

$$\frac{V \times 22 \times N}{W}\left[\text{mol}\,(CO_2)\,\text{Kg}^{-1}\,(\text{leaf fresh mass})\text{s}^{-1}\right]$$

where V = difference in vol of HCl in Control and the sample

22 = Equivalent wt of CO_2

N = Normality of HCl

W = Weight of tissue used

Chlorophyll Estimation

Chlorophyll was estimated following the method of Mac Kinney (1941). Fresh leaves 0.1g were homogenised in a mortar in the presence of sufficient quantity of 80% acetone. The extract was filtered and supernatant collected in the volumetric flask. The process was repeated thrice and each time supernatant was collected in the same flask. Finally the volume was made upto 10 ml with 80% acetone. 5 ml sample of chlorophyll extract was transferred to a cuvette and absorbance was read at 645 and 663 nm on spectrophotometer. The following formula was used to calculate chlorophyll contents.

$$\text{Total Chlorophyll (mg g - 1)} = [20.2\,(D645) + 8.02\,(D663)] \times \frac{V}{1000 \times W}$$

where V = Volume of solution

W = Weight of tissue used for extraction of pigments

Carotenoid Estimation

However for carotenoids were estimated following the method of Machlachlan and Zalik (1963). For this 100 mg fresh leaves were homogenized in 80% acetone, after filtering it the volume was maintained upto 10 ml. Readings were taken at 400 nm and 510 nm and carotenoids were calculated by the formula

$$\text{Carotenoids} = \frac{(7.6) \times (480) - 1.49 \times (510)}{d \times 1000 \times W}$$

where W = Weight of the sample

d = Length of light path

3.11 Leaf Analysis

Dried leaf samples collected at vegetative, flowering and fruiting stages were used for the estimation of N, P an K contents. The details of estimation procedures are as follows.

Digestion of Leaf Samples

Healthy leaves from oven dried plant material collected at different growth stages were used for N, P and K estimation. The dried leaves were removed and powdered with mortar and pestle and passed through a 72 mm mesh screen. 50 mg of this oven dried powder from each replicate was transferred to a 50 ml kjeldhal flask to which 2 ml sulphuric acid was added. The contents of the flask were heated on temperature controlled assembly for about 2 hours, to allow complete reduction of nitrate present in the plant material by organic matter itself. As a result the content of the flask turned black. After cooling the flask for about 15 minutes, 0.5 ml 30% H_2O_2 was added drop by drop and the solution was heated again till the colour changed from black to light yellow. Again after cooling for 30 minutes additional 3-4 drops of 30% H_2O_2 was added and then again heated for about 15 minutes, the process was repeated till the contents of the flask became colourless. The peroxide digested material was then transferred to 50 ml volumetric flask with three washings with

DDW. The volume of the flask was maintained up to the mark for the estimation of N, P and K contents.

Nitrogen Estimation

It was estimated according to Lindner (1944). 10 ml aliquot of peroxide digested material was taken in a 50 ml conical flask. To this 2 ml 2.5 N NaOH and 1 ml 10% sodium silicate solution was added, it was neutralized with excess of acid. Volume was made upto the mark with DDW. In a 10 ml graduated test tube, 5 ml of this solution was taken and 0.5 ml Nessler's reagent (Appendix, iii) was added. The final volume was made with DDW. The contents of tube were allowed to stand for 5 minutes for maximum colour development. The solution was transferred to a colorimetric tube and optical density (OD) was read at 525 nm with the help of spectrophotometer.

Standard Curve for Nitrogen

50 mg ammonium sulphate was dissolved in 1 litre DDW. From this solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml was pipetted to 10 different test tubes of 10 ml volume. The solution in each test tube was diluted to 5 ml with DDW and after adding 0.5 ml Nessler's reagent volume of each tube was maintained upto the mark with DDW. The optical density was read at 525 nm on spectrophotometer. A blank was also run with each set of determination. Standard curve was plotted using different concentrations of ammonium sulphate solution versus optical density (OD) and nitrogen of the sample was determined with the help of this standard curve.

Phosphorus Estimation

The method of Fiske and Subba Row (1925) was used to estimate the total phosphorus in the digested material. 5 ml aliquot was taken in a 10 ml graduated test tube and 1 ml molybdic acid reagent (2.5%) (Appendix, iii) was carefully added, followed by the addition of 0.4 ml (1-amino-2-naphthol-4-sulphonic acid) (Appendix, i). Addition of this turned the colour of the contents blue. Volume was maintained upto 10 ml with DDW. The solution was shaken for 5 minutes for maximum colour development and subsequently transferred to a colorimetric tube. The optical density was read at 620 nm on spectrophotometer. A blank was also run simultaneously.

Standard Curve for Phosphorus

351 mg monobasic dihydrogen orthophosphate was dissolved in sufficient DDW to which 10 ml 10N H_2SO_4 was added and final volume was made upto 1000 ml with DDW. From this solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml was taken in ten different graduated test tubes. The solution in each test tube was diluted to 5 ml with DDW. In each tube 1 ml molybdic acid reagent and 0.4 ml 1-amino-2 napthol-4-sulphonic acid was added and the final volume was made upto 10 ml with DDW in all the test tubes. After 5 minutes, optical density was read at 620 nm on spectrophotometer. A blank was also run with each set of determination. Standard curve was plotted using different dilutions of potassium dihydrogen orthophosphate solution versus optical density with the help of standard curve, the amount of phosphorus present in the sample was determined.

Potassium Estimation

Potassium was estimated with the help of flame photometer. 10 ml aliquot was taken and it was read by using the filter for potassium. A blank was also run side by side with each set of determination. The readings were compared with a calibration curve plotted against known dilutions of standard potassium chloride solution.

Standard Curve for Potassium

1.91 g potassium chloride was dissolved in 100 ml DDW of which 1 ml solution was diluted to 1000 ml. From this resulting solution of 10 ppm 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ml solution was transferred to 10 vials separately. The solution in each vial was diluted to 10 ml with DDW. The dilute solutions of each vial was run separately. A blank was also run with each set of determination. Standard curve was prepared using different dilutions of potassium chloride solution versus reading on the scale of galvanometer. The amount of potassium present in the sample was determined with the help of standard curve.

3.12 Yield Parameters

Six plants left after three samplings from each treatment were taken at the time of harvest and following yield characteristics were observed.

1. Siliqua length (cm) plant^{-1}
2. Siliqua number plant^{-1}
3. Seeds siliqua^{-1}

4. 1000 seed weight (g)
5. Seed yield plant^{-1}
6. Plant biomass

Total seeds threshed out of dried plant samples of each treatment were cleaned and allowed to dry under the sun for a few hours and their weight was recorded. Total biomass was recorded after drying the whole plant in the sun.

3.13 Seed Analysis

The seeds collected at harvest were chemically analysed for oil and protein content. The seed samples of each treatment were dried and ground to get powdered seed meal.

Seed Oil

10g of powdered seed meal was transferred to a soxhlet apparatus and sufficient quantity of petroleum ether was added. The apparatus was kept on hot water bath running at 60°C for about 6 hours for complete extraction of the oil. The petroleum ether from the extracted oil was evaporated after sometime. The extracted oil was expressed as percentage by mass of the seeds and was calculated by the following formula.

$$\text{Oil content \%} = \frac{mo}{ms} \times 100$$

where mo = Sum of mass of oil; ms = mass of seed sample

Oil Yield

The oil yield was calculated on the basis of oil content and seed yield.

Estimation of Seed Protein

In addition to oil, the seed of Indian mustard also contain protein from which oil cakes are made and are commonly fed to livestock. The method of Lowry *et al.* (1951) was followed. 50 mg oven dried seed powder was transferred into glass centrifuge tube, to which 5 ml of 5% trichloro-acetic acid was added. The solution was allowed to stand for 30 minutes at room temperature with thorough shaking for the complete precipitation of the protein. The material was centrifuged at 4000 rpm for 10 minutes and the supernatant was discarded. 5 ml of 1N sodium hydroxide was added to the residue and mixed well. It was left for 30 minutes on water bath at 80°c so that all the precipitated protein may completely get dissolved. After cooling for 15 minutes the mixture was centrifuged again at 4000 rpm for 15 minutes and the supernatant containing protein fraction together with three washings with 1N NaOH was collected in 25 ml volumetric flask. Volume was made upto the mark with 1N NaOH and used for the estimation of protein. 1 ml of sodium hydroxide extract was transferred to 10 ml test tube and 5 ml reagent–B (Appendix, iv) was added. The solution was mixed well and allowed to stand for 10 minutes at room temperature. 0.5 ml Folin's phenol reagent (Appendix, ii) was added rapidly with immediate mixing. The blue colour developed was left for 30 minutes for maximum colour development, absorbance was read at 660 nm. A blank containing DDW, reagent–B and Folin's phenol reagent was simultaneously run with each sample. The protein contents were calculated by comparing the optical density of each sample with calibration curve plotted by taking known graded dilutions of standard solution of Bovine serum albumin (Fraction – V) and seed protein contents were expressed in terms of percentage on dry weight basis.

Standard Curve for Protein

50 mg bovine serun albumin (Fraction -V) was dissolved in 50 ml DDW, of which 10 ml solution was diluted to 50 ml. 1 ml of this solution contains 200 μg protein. From this 0.2, 0.4, 0.6, 0.8 and 1.0 ml solution was transferred to 5 test tubes separately. The solution in each test tube was diluted to 1 ml with DDW. A blank of 1 ml DDW was also run with each set of determination. 5ml reagent B to each tube including blank was mixed well and allowed to stand for 10 minutes. To this solution 0.5 ml Folin's phenol reagent was added and mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour developed was read at 660 nm.

3.14 Estimation of Heavy Metals

Since crop received effluent of electroplating industrial waste mixed with sewage therefore, it was decided to estimate Cd, Ni, Cr, Pb heavy metals also in soil, water and seeds (Tables 6,68) using atomic absorption spectrophotometer.

Preparation of Samples for Analysis

Soil Samples

1g of soil sample was taken in a conical flask. To this 10 ml of nitric acid was added. It was placed on hot plate. After 12 hours of digestion, 5 ml of perchloric acid was added for complete digestion. After cooling it was filtered and volume was made upto 100 ml with DDW. After filtering through Whatman filter paper No. 42 it was stored in polyethene bottles and analysed on GBS 902 double beam atomic absorption spectrophotometer.

Water Sample

20 ml water sample was taken in a conical flask. To this 10 ml of nitric acid was added. It was placed on a hot plate for digestion. After complete digestion, total volume was made upto 100 ml with DDW. It was stored in polyethene bottles after filtering through whatman filter paper No. 42. The samples were read at GBS 902 double beam atomic absorption spectrophotometer.

Seed Samples

500 mg of grain powder was taken in a conical flask. After adding 10 ml nitric acid, it was placed for digestion. After complete digestion it was filtered, final volume was made upto 100 ml. Then after filtering through whatman filter paper No 42 readings were taken on GBS 902 double beam atomic absorption spectrophotometer.

EXPERIMENTAL RESULTS

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EXPERIMENTAL RESULTS

Chapter 4

EXPERIMENTAL RESULTS

4.1 EXPERIMENT – I

In this complete randomized block design pot experiment, conducted on *Brassica juncea* var. Varuna, comparative effect of two concentrations of city wastewater (WW) and GW with four levels of nitrogen was studied. Most of the data were found significant and briefly described below.

4.1.1 Growth Parameters

Plant height, leaf number, leaf area, plant fresh weight and plant dry weight were studied at vegetative, flowering and fruiting stages.

4.1.1.1 Plant Height

It was evident from the table 8 that WW significantly increased the height of the plants. An increase of 10.06%, 15.73%, 12.80% and 6.19%, 9.98%, 9.29% was shown at three growth stages by 100%WW and 50%WW respectively. Both concentrations were at par at vegetative stage, whereas at flowering and fruiting the two wastewater treatments performed differently. With the increasing doses of nitrogenous fertilizer, plant height also increased linearly giving maximum value at N_{120} at vegetative and flowering stages. An increase of 44.28%, 53.05% and 42.37% was recorded by N_{120} over No at the three stages. Rest of the doses of nitrogen proved deficient for this character. Interaction was non significant at vegetative and fruiting stages. Regarding plant response, height of plants increased from vegetative to fruiting stage.

4.1.1.2 Leaf Number Plant^{-1}

WW produced more number of leaves over GW (Table 9). 100%WW recorded the maximum increase of 14.66%, 12.91% and 14.06%, followed by 50%WW where the percentage increase was 7.93%, 11.03% and 6.42% over GW at vegetative flowering and fruiting stages. At flowering 100%WW and 50%WW showed at par values. Leaf number was also increased with the application of fertilizer. Contrary to plant height, N_{80} gave the optimum value as it was at par with N_{120} and the former recorded an increase of 32.97%, 39.83% and 36.80% over N_0

Table 8. Effect of GW, 50% WW and 100% WW on plant height (cm) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	26.70	28.40	29.10	28.07	43.80	48.10	50.60	47.50
N_{40}	30.80	33.30	34.80	32.97	53.00	58.80	62.20	58.00
N_{80}	36.00	38.80	39.30	38.03	65.80	72.10	75.30	71.07
N_{120}	38.90	40.10	42.50	40.50	67.00	73.50	77.60	72.70
Mean	33.10	35.15	36.43		57.40	63.13	66.43	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	58.60	62.50	64.90	62.00
N_{40}	66.70	74.00	77.30	72.67
N_{80}	81.00	88.80	90.30	86.70
N_{120}	82.10	89.90	92.80	88.27
Mean	72.10	78.80	81.33	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	1.527	0.800	1.466
Nitrogen	1.763	0.925	1.694
Interaction	NS	1.604	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 9. Effect of GW, 50% WW and 100% WW on leaf number plant^{-1} of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	13.96	14.74	15.25	14.65	25.26	27.62	27.80	26.89
N_{40}	16.08	16.69	17.20	16.66	29.00	31.50	32.38	30.96
N_{80}	17.14	19.81	21.50	19.48	34.00	38.66	40.13	37.60
N_{120}	18.84	20.03	21.78	20.22	35.06	39.14	38.92	37.71
Mean	16.51	17.82	18.93		30.83	34.23	34.81	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
N_0	21.68	23.14	23.82	22.88				
N_{40}	25.04	27.21	28.01	26.75	Water	0.721	1.632	0.950
N_{80}	29.13	30.76	34.00	31.30	Nitrogen	0.835	1.885	1.101
N_{120}	30.00	31.54	34.88	32.14	Interaction	NS	NS	NS
Mean	26.46	28.16	30.18					

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

where as N_{40} proved deficient dose, while the highest dose, N_{120} was at luxury consumption. Leaf number increased only upto flowering stage, after which it decreased.

4.1.1.3 Leaf Area Plant^{-1}

Application of both the concentrations of wastewater was responsible for increasing the leaf area also at vegetative and flowering stages, but at fruiting it proved non significant (Table 10). An increase of 11.34% and 12.31% by 100%WW and 7.71 and 9.16% by 50%WW was recorded over GW. Since N_{80} was statistically at par with N_{120} at vegetative, flowering and fruiting stages, thus proved optimum and registered an increase of 38.64%, 40.98% and 45.91% over N_0. Among all the interactions 100%×N_{80} gave the optimum value and it was at par with 100%×N_{120}, which in turn was also at par with 50%×N_{120} at vegetative stage. Like leaf number, leaf area also increased from vegetative to flowering stage, after which it was decreased.

4.1.1.4 Fresh Weight Plant^{-1}

It may be noted from the table 11 that higher fresh weight was attained by the plants grown with WW. An increase of 14.31%, 14.89%, 12.67% and 9.81%, 12.04%, 10.28% was recorded with 100%WW and 50%WW respectively at the three successive stages of growth, although at flowering at par values were given by the two concentration of WW. Like leaf number and leaf area, N_{80} again proved optimum dose as it was at par with N_{120} at all the three successive stages studied and it marked an increase of 37.67%, 38.83% and 30.99% over N_0. Among interactions, 100%×N_{80} proved optimum and recorded an increase of 54.19%, 56.07% and 44.03% over GW×N_0, however it was at par with 50%×N_{120} and 100%×N_{120}. Among other interactions, GW×N_{80} was at par with GW×N_{120} on the one hand and on the other it was at par with 100%×N_{40}, thus showing the utility of wastewater where lower dose of nitrogen (N_{40}) proved equally effective when applied with 100% wastewater. An increasing trend like the plant height was observed in the plant fresh weight, upto the last stage. Percentage increase was more from vegetative to flowering stage.

Table 10. Effect of GW, 50% WW and 100% WW on leaf area (cm^2 $plant^{-1}$) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	277.28	284.32	290.64	284.08	364.61	395.77	404.61	388.33
N_{40}	308.93	332.18	344.40	328.50	425.55	465.17	480.18	456.97
N_{80}	360.33	400.52	420.67	393.84	507.07	560.23	575.11	547.47
N_{120}	374.75	406.16	415.43	398.78	519.12	561.62	580.15	553.63
Mean	330.32	355.80	367.79		454.09	495.70	510.01	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	318.76	348.56	357.00	341.44
N_{40}	380.19	421.45	433.97	411.87
N_{80}	455.41	508.02	531.12	498.18
N_{120}	467.40	512.32	513.74	497.82
Mean	405.44	447.59	458.96	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	5.384	5.814	NS
Nitrogen	6.217	6.713	12.51
Interaction	10.768	11.628	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 11. Effect of GW, 50% WW and 100% WW on plant fresh weight (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	8.36	8.88	9.04	8.76	14.50	15.48	16.06	15.35
N_{40}	9.32	10.30	10.76	10.13	16.42	18.66	19.06	18.05
N_{80}	11.00	12.28	12.89	12.06	19.30	22.00	22.63	21.31
N_{120}	11.27	12.43	13.00	12.23	19.90	22.40	22.80	21.70
Mean	9.99	10.97	11.42		17.53	19.64	20.14	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	23.37	24.44	24.71	24.17
N_{40}	25.34	28.50	28.45	27.43
N_{80}	28.93	32.40	33.66	31.66
N_{120}	29.70	33.04	34.12	32.29
Mean	26.84	29.60	30.24	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.241	0.648	0.619
Nitrogen	0.277	0.748	0.715
Interaction	0.482	1.296	1.238

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

4.1.1.5 Dry Weight Plant^{-1}

WW resulted in higher dry matter accumulation as it differed critically from GW (Table 12). Percentage increase with 100%WW was 10.53%, 12.41%, 11.89% over GW, whereas with 50%WW it was 7.12%, 6.99% and 7.03% at vegetative, flowering and fruiting stages. At first stage, 100%WW and 50%WW gave at par values. Like leaf number and leaf area, for dry weight also N_{80} proved optimum dose by recording an increase of 38.83%, 33.27% and 33.37% over N_0. Interaction was significant at fruiting stage only, where 100%×N_{80} gave the maximum value by marking an increase of 46.92% over GW×N_0. Further, GW×N_{80} was also at par with GW×N_{120} on one hand, whereas on the other with 100%×N_{40}. Like plant height and fresh weight, dry weight also showed an increasing trend with the increase in the age of the plants. However, increase was more from vegetative to flowering stage.

4.1.2 Physiological Parameters

Total chlorophyll content, carotenoids, nitrate reductase activity, carbonic anhydrase activity, leaf nitrogen, phosphorus and potassium content were also studied at vegetative, flowering and fruiting stages.

4.1.2.1 Total Chlorophyll Content

WW increased the total chlorophyll content of the leaves (Table 13). 100%WW recorded an increase of 13.72%, 14.42% and 15.85% over GW, with 50%WW the percentage increase was 9.80%, 9.13% and 15.30%. At fruiting, 50%WW was at par with 100%WW. N_{80} gave values at par to N_{120} and marked an increase of 32.85%, 36.41% and 39.76% over N_0. Among various interactions, 100%×N_{80} was the best combination at flowering and fruiting stages, an increase of 56.25% and 57.50% respectively was marked by this dose over GW×N_0. At both the stages, GW×N_{80} was at par with GW×N_{120} on the one hand, whereas on the other with 100%×N_{40} again proving the utility of wastewater in nitrogen economy. Chlorophyll content increased from vegetative to flowering stage and then decreased at fruiting.

4.1.2.2 Carotenoids

From table 14 an increase of 10.63%, 8.73% and 10.40% may be noted with 100%WW application. However, at par values were shown by 100% and 50% wastewater at all the stages studied. Contrary to most of the parameters, carotenoid

Table 12. Effect of GW, 50% WW and 100% WW on plant dry weight (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	2.62	2.73	2.84	2.73	4.82	5.04	5.20	5.02
N_{40}	3.05	3.38	3.40	3.28	5.38	5.76	6.02	5.72
N_{80}	3.55	3.81	4.00	3.79	6.21	6.70	7.15	6.69
N_{120}	3.68	3.93	4.05	3.89	6.48	6.98	7.34	6.93
Mean	3.23	3.46	3.57		5.72	6.12	6.43	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	7.80	8.22	8.43	8.15
N_{40}	8.75	9.28	9.95	9.33
N_{80}	10.16	11.00	11.46	10.87
N_{120}	10.30	11.11	11.54	10.98
Mean	9.25	9.90	10.35	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.193	0.223	0.161
Nitrogen	0.223	0.257	0.185
Interaction	NS	NS	0.319

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 13. Effect of GW, 50% WW and 100% WW on total chlorophyll content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	1.30	1.38	1.43	1.37	1.76	1.86	1.91	1.84
N_{40}	1.41	1.61	1.65	1.56	1.98	2.17	2.23	2.13
N_{80}	1.68	1.84	1.94	1.82	2.25	2.53	2.75	2.51
N_{120}	1.73	1.88	1.94	1.85	2.34	2.53	2.62	2.50
Mean	1.53	1.68	1.74		2.08	2.27	2.38	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	1.60	1.68	1.70	1.66
N_{40}	1.76	1.87	1.90	1.84
N_{80}	1.94	2.50	2.52	2.32
N_{120}	2.03	2.38	2.35	2.25
Mean	1.83	2.11	2.12	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.051	0.055	0.065
Nitrogen	0.062	0.062	0.075
Interaction	NS	0.108	0.123

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

content increased with the increase of the fertilizer dose, thus N_{120} attained maximum values of 34.81%, 31.14% and 33.64% over N_0. Interaction was significant at vegetative stage only where 100%×N_{80} was the optimum dose as it marked an increase of 43.13% over GW×N_0, but was at par with 100%×N_{120}, 50%×N_{120} and 50%×N_{80} also. Carotenoids followed the trend of the total chlorophyll content, which increased upto flowering only.

4.1.2.3 Nitrate Reductase Activity (NRA)

City wastewater also increased the enzymatic activity (Table 15). An increase of 11.11%, 13.35%, 12.35% and 7.80%, 10.43%, 9.98% was recorded with 100%WW and 50%WW at the three stages studied. Among various doses of nitrogen, N_{80} again proved optimum and recorded an increase of 39.37%, 36.22% and 39.78% over N_0. Like leaf number, leaf area, chlorophyll content and carotenoids, NRA also increased upto flowering stage and then decreased.

4.1.2.4 Carbonic Anhydrase (CA) Activity

Carbonic anhydrase activity was also enhanced by WW irrigation thus higher percentage of 8.48%, 8.77% and 12.88% was marked with 100%WW (Table 16). At vegetative and fruiting stages, 100%WW was at par with 50%WW. N_{120} was at par with N_{80} and the percentage increase was 32.28%, 32.71% and 28.53% by the latter dose. Like NRA, CA also increased upto flowering stage and then it decreased.

4.1.2.5 Leaf Nitrogen Content

100%WW performed differently and differed significantly from 50% at vegetative and fruiting, whereas at flowering stage both concentrations gave at par values (Table 17). An increase of 8.66%, 9.80%, 9.28% and 4.72%, 6.92% and 5.39% was shown by 100%WW and 50%WW respectively. N_{80} was at par with N_{120} at every stage, thus proved the optimum dose and again showed the luxury consumption of nitrogen when applied as N_{120}. Optimum dose recorded an increase of 29.25%, 30.10% and 32.41% at three growth stages. Interaction was non significant. Nitrogen content showed a decreasing trend with the increase in age of the plants.

4.1.2.6 Leaf Phosphorus Content

100%WW as well as 50%WW significantly increased the leaf phosphorus content over GW (Table 18). 100%WW recorded an increase of 12.30%, 10.85% and

Table 14. Effect of GW, 50% WW and 100% WW on carotenoid content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	0.415	0.421	0.449	0.428	0.570	0.618	0.632	0.607
N_{40}	0.461	0.510	0.521	0.497	0.658	0.721	0.749	0.709
N_{80}	0.536	0.580	0.594	0.570	0.750	0.782	0.795	0.776
N_{120}	0.545	0.585	0.600	0.577	0.770	0.805	0.813	0.796
Mean	0.489	0.524	0.541		0.687	0.732	0.747	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
N_0	0.508	0.542	0.554	0.535				
N_{40}	0.570	0.610	0.604	0.595	Water	0.021	0.015	0.017
N_{80}	0.640	0.708	0.726	0.691	Nitrogen	0.021	0.017	0.020
N_{120}	0.664	0.734	0.747	0.715	Interaction	0.036	NS	NS
Mean	0.596	0.649	0.658					

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 15. Effect of GW, 50% WW and 100% WW on nitrate reductase activity (μ mol g^{-1} leaf fresh weight h^{-1}) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	283.01	295.23	304.40	294.21	336.18	360.18	370.11	355.49
N_{40}	318.87	340.10	361.53	340.17	393.08	420.27	437.36	416.90
N_{80}	380.60	418.28	431.23	410.04	438.02	498.23	516.48	484.24
N_{120}	385.62	421.18	423.01	409.94	450.16	507.48	509.37	489.00
Mean	342.02	368.70	380.04		404.36	446.54	458.33	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
N_0	311.22	330.34	337.99	326.52				
N_{40}	350.96	383.91	396.50	377.12	Water	7.83	8.63	7.66
N_{80}	418.92	474.10	476.21	456.41	Nitrogen	9.05	9.97	8.78
N_{120}	426.16	469.24	482.64	459.35	Interaction	NS	NS	NS
Mean	376.81	414.40	423.33					

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 16. Effect of GW, 50% WW and 100% WW on carbonic anhydrase activity [mol (CO_2) kg^{-1} (leaf fresh mass) s^{-1}] of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	2.72	2.90	2.93	2.85	3.54	3.84	3.90	3.76
N_{40}	3.10	3.43	3.50	3.34	4.09	4.57	4.61	4.42
N_{80}	3.60	3.82	3.89	3.77	4.80	4.91	5.26	4.99
N_{120}	3.76	3.92	3.98	3.89	4.88	4.96	5.08	4.97
Mean	3.30	3.52	3.58		4.33	4.57	4.71	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	3.30	3.41	3.48	3.40
N_{40}	3.56	3.63	3.76	3.65
N_{80}	3.81	4.65	4.64	4.37
N_{120}	3.94	4.51	4.60	4.35
Mean	3.65	4.05	4.12	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.148	0.123	0.417
Nitrogen	0.171	0.142	0.497
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 17. Effect of GW, 50% WW and 100% WW on leaf nitrogen content (%) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	3.21	3.36	3.48	3.35	2.95	3.10	3.22	3.09
N_{40}	3.60	3.80	3.98	3.79	3.34	3.51	3.66	3.50
N_{80}	4.14	4.36	4.50	4.33	3.74	4.17	4.16	4.02
N_{120}	4.28	4.45	4.59	4.44	3.85	4.05	4.21	4.04
Mean	3.81	3.99	4.14		3.47	3.71	3.81	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	2.78	2.90	3.02	2.90
N_{40}	3.16	3.38	3.50	3.35
N_{80}	3.65	3.86	4.00	3.84
N_{120}	3.78	3.95	4.07	3.93
Mean	3.34	3.52	3.65	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.113	0.123	0.113
Nitrogen	0.131	0.141	0.131
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

11.79%, whereas an increase of 8.81%, 7.89%, 8.00% was noted with 50%WW at three stages respectively. Both the concentrations of WW gave at par values at flowering stage. For phosphorus content N_{120} proved the optimum dose, showing the synergistic effect and recorded an increase of 40.80%, 41.47% and 40.43%. At par values were shown by N_{120} and N_{80} only at flowering stage. Like nitrogen content, phosphorus content of leaves also showed a decreasing trend from vegetative to fruiting stage.

4.1.2.7 Leaf Potassium Content

It was clear from the table 19 that potassium content of wastewater irrigated plants was higher than the plants irrigated with GW. At vegetative stage both the concentrations performed differently, but at the other two stages 100%WW gave values at par to 50%WW. An increase of 12.53%, 11.51% and 11.87% was shown by 100%WW. N_{80} proved optimum being at par with N_{120} at vegetative and fruiting stages but at flowering stage maximum values were recorded, with N_{120} showing its luxury consumption. An increase of 38.07%, 33.96% and 33.00% was marked with optimum dose. Interaction was non significant. Potassium content also decreased consistently with the increase in age of the plants. Among three nutrients, K contents were more followed by nitrogen and phosphorus.

4.1.3 Yield and Quality Parameters

Yield and quality parameters i.e. siliqua length, number of siliqua plant^{-1}, seeds siliqua^{-1}, 1000 seed weight, seed yield plant^{-1}, plant biomass, oil content, oil yield, seed protein and heavy metals of seeds were observed at harvest and described as under.

4.1.3.1 Siliqua Length

No significant increase in the length of siliqua was observed with wastewater application (Table 20). However, fertilizer dose helped in increasing this parameter significantly. N_{120} gave the optimum value by marking an increase of 27.84% over control followed by N_{80} and N_{40} where the percentage increase was 22.89 and 11.36 respectively.

Table 18. Effect of GW, 50% WW and 100% WW on leaf phosphorus content (%) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	0.452	0.474	0.493	0.473	0.410	0.440	0.453	0.434
N_{40}	0.511	0.558	0.576	0.548	0.480	0.519	0.536	0.512
N_{80}	0.594	0.660	0.683	0.646	0.558	0.603	0.620	0.594
N_{120}	0.621	0.681	0.696	0.666	0.580	0.624	0.637	0.614
Mean	0.545	0.593	0.612		0.507	0.547	0.562	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	0.397	0.413	0.428	0.413
N_{40}	0.446	0.476	0.489	0.470
N_{80}	0.518	0.574	0.596	0.563
N_{120}	0.540	0.590	0.610	0.580
Mean	0.475	0.513	0.531	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.015	0.022	0.012
Nitrogen	0.017	0.026	0.014
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 19. Effect of GW, 50% WW and 100% WW on leaf potassium content (%) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	3.18	3.31	3.45	3.31	2.98	3.21	3.36	3.18
N_{40}	3.59	3.90	4.07	3.85	3.45	3.72	3.82	3.66
N_{80}	4.20	4.68	4.82	4.57	3.95	4.36	4.48	4.26
N_{120}	4.34	4.75	4.89	4.66	4.22	4.50	4.60	4.44
Mean	3.83	4.16	4.31		3.65	3.95	4.07	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
N_0	2.86	3.03	3.10	3.00
N_{40}	3.23	3.38	3.50	3.37
N_{80}	3.64	4.22	4.10	3.99
N_{120}	3.75	4.14	4.37	4.09
Mean	3.37	3.69	3.77	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.088	0.148	0.123
Nitrogen	0.102	0.158	0.142
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

4.1.3.2 Siliqua Number Plant^{-1}

City wastewater proved effective for significant increase in the siliquae (Table 20) whereas GW was least effective, thus 100%WW increased it by 10.31% whereas with 50%WW application the percentage increase was 6.28. Among various fertilizer treatments, N_{80} gave the optimum value, as it was at par with N_{120} and recorded an increase of 27.24% over control which was 14.67% more than the deficient dose (N_{40}) of nitrogen. From various interactions, 100%×N_{80} proved optimum as it was at par with 100%×N_{120} and registered an increase of 39.05% over GW×N_0. Among other interactions, GW×N_{80} was at par with GW×N_{120} on one hand but on the other it was at par with 100%×N_{40}, further GW×N_0 was at par with 50%×N_0 on one hand and on the other with GW×N_{40}.

4.1.3.3 Seeds Siliqua^{-1}

Table 20 showed that 100%WW as well as 50%WW proved beneficial in increasing the number of seeds per siliqua by marking an increase of 5.07% and 2.67% respectively over GW. Fertilizer dose N_{80} was at par with N_{120} and marked an increase of 9.77% over N_0, whereas an increase of 4.11% was shown by N_{40}. Interaction was non significant.

4.1.3.4 1000 Seed Weight

Effect of city wastewater was non-significant for 1000 seed weight (Table 21). However the fertilizer treatment effect was significant thus N_{80} registered an increase of 9.17% over N_0 and it was at par with N_{120} confirming the effectiveness of N_{80} dose. The lowest fertilizer dose could enhance only 3.12% 1000 seed weight when compared with N_0.

4.1.3.5 Seed Yield Plant^{-1}

Application of wastewater enhanced the seed yield over GW (Table 21) showing the cumulative effect of enhanced growth and yield parameters due to WW. An increase of 14.23% and 9.00% was recorded with 100%WW and 50%WW respectively over GW in terms of seed production. Like wise, as observed in most of the parameters fertilizer dose N_{80} proved optimum as it was at par with N_{120} and recorded an increase of 37.32% over N_0. It may be noted here that an increase of 15.46% in seed yield was also recorded under N_{40} over control. Among interactions,

Table 20. Effect of GW, 50%WW and 100%WW on siliqua length (cm), siliqua number plant^{-1} and seeds siliqua^{-1} of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Siliqua length				Siliqua number			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	5.31	5.44	5.62	5.46	92.82	96.56	98.18	95.85
N_{40}	5.62	6.25	6.38	6.08	100.44	107.14	111.51	106.36
N_{80}	6.40	6.82	6.90	6.71	113.96	122.86	129.07	121.96
N_{120}	6.90	7.00	7.03	6.98	117.44	124.75	129.70	123.96
Mean	6.06	6.38	6.48		106.17	112.83	117.11	

	Seeds siliqua^{-1}			
	GW	50%WW	100%WW	Mean
N_0	13.75	14.23	14.38	14.12
N_{40}	14.41	14.74	14.95	14.70
N_{80}	15.01	15.53	15.96	15.50
N_{120}	15.20	15.40	16.02	15.54
Mean	14.59	14.98	15.33	

CD at 5%	Siliqua length	Siliqua number	Seeds siliqua^{-1}
Water	NS	1.861	0.289
Nitrogen	0.261	2.149	0.334
Interaction	NS	3.722	NS

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

Table 21. Effect of GW, 50%WW and 100%WW on 1000-seed weight (g), seed yield (g $plant^{-1}$) and biomass (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	1000-seed weight				Seed yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	5.33	5.48	5.54	5.45	6.88	7.18	7.48	7.19
N_{40}	5.52	5.63	5.71	5.62	7.66	8.48	8.73	8.29
N_{80}	5.80	5.98	6.07	5.95	8.95	10.00	10.64	9.86
N_{120}	5.90	6.05	6.11	6.02	9.37	10.16	10.70	10.08
Mean	5.64	5.78	5.86		8.22	8.96	9.39	

	Biomass				CD at 5%			
	GW	50%WW	100%WW	Mean		1000-seed weight	Seed yield	Biomass
N_0	11.35	12.00	12.38	11.91				
N_{40}	12.80	13.81	14.30	13.64	Water	NS	0.201	0.163
N_{80}	14.56	16.09	16.41	15.69	Nitrogen	0.232	0.232	0.188
N_{120}	14.86	16.28	16.57	15.90	Interaction	NS	0.399	0.326
Mean	13.39	14.55	14.92					

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

100%×N_{80} was the optimum combination. It recorded an increase of 54.65% over GW×N_0 and was at par with 100%×N_{120}. Among other interactions 100%×N_{40} was at par with 50%×N_{40} on one hand and on the other hand with GW×N_{80}. Also 100%×N_0 was at par with GW×N_{40}, proving the importance of wastewater in saving inorganic fertilizers on one hand and utilizing the wastewater in agricultural productivity on the other.

4.1.3.6 Plant Biomass

As evident from the table 21 city waste water fed plants showed more biomass accumulation than those irrigated with GW. An increase of 11.43% was shown by 100%WW whereas 50%WW marked an increase of 8.66% over GW. For this parameter also N_{120} proved comparatively better dose over other doses and among interactions 100%×N_{80} proved superior, however its value was at par with 100%×N_{120}, 50%×N_{80} on the on hand and on the other with 50%×N_{120}, further GW×N_{80} was also at par with GW×N_{120} and 100%×N_{40}.

4.1.3.7 Oil Content

Increased oil contents were also noted in plants irrigated with wastewater (Table 22). 50%WW gave values which were at par to 100%WW as well as GW showing no adverse effect of wastewater on this important parameter. An increase of 3.46% was shown by 100%. Nitrogenous fertilizer increased oil content upto 40 kg ha^{-1} only after which it started decreasing. An increase of 4.53% was shown by N_{40}. Interactions were non-significant.

4.1.3.8 Oil Yield Plant^{-1}

For oil yield, 100% as well as 50%WW performed differently and surpassed GW (Table 22). Former marked an increase of 18.31%, whereas an increase of 11.63% was shown by the latter. Among different doses of nitrogen N_{80} was the optimum, recording an increase of 38.28% over N_0. The treatment N_{120} gave at par values to N_{80} showing the wasteful consumption, whereas N_{40} proved deficient. Among various interactions, 100%×N_{80} was the best combination as it marked as increase of 58.68% over GW×N_0 and gave value equal to 100%×N_{120}. Further more, GW × N_{120} gave value at par to 100%×N_{40} and 50%×N_{40}, also at par values were

Table 22. Effect of GW, 50% WW and 100% WW on oil content (%), oil yield (g $plant^{-1}$) and seed protein content (%) of *Brassica juncea* cv. Varuna with four levels of nitrogen.

Treatments	Oil content				Oil yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
N_0	41.82	42.00	42.76	42.19	2.88	3.02	3.20	3.03
N_{40}	42.91	44.27	45.12	44.10	3.29	3.75	3.94	3.66
N_{80}	41.61	42.69	42.95	42.42	3.72	4.27	4.57	4.19
N_{120}	41.32	42.46	42.66	42.15	3.87	4.31	4.56	4.25
Mean	41.92	42.86	43.37		3.44	3.84	4.07	

	Seed protein content				CD at 5%			
	GW	50%WW	100%WW	Mean		Oil content	Oil yield	Seed Protein content
N_0	21.20	22.00	22.41	21.87				
N_{40}	22.23	23.28	23.76	23.09	Water	1.38	0.11	0.235
N_{80}	23.61	23.96	23.38	23.65	Nitrogen	1.63	0.13	0.271
N_{120}	23.85	24.30	24.55	24.23	Interaction	NS	0.24	NS
Mean	22.72	23.39	23.53					

N.B.: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{20} and K_{20} in kg ha^{-1} was also applied basally.

shown by $100\% \times N_0$ and $GW \times N_{40}$ highlighting the utility of wastewater for higher oil yield production and wasteful use of fertilizer dose in presence of WW.

4.1.3.9 Seed Protein Content

Protein content of seeds obtained with wastewater was also enhanced (Table 22). Both 100%WW and 50%WW gave significantly at par values and an increase of 3.57% was marked by the former over GW. Among various doses of nitrogen, N_{120} gave the maximum value by recording an increase of 10.79% over N_0. Interaction was non significant.

4.2 EXPERIMENT -II

In this pot experiment also *Brassica juncea* var. Varuna was irrigated under the same concentration of wastewater along with GW as control. In addition to this phosphatic fertilizer was tested in four different levels.

4.2.1 Growth Parameters

Like experiment I, plant height, leaf number, leaf area, plant fresh weight and plant dry weight were observed at vegetative, flowering and fruiting stages. Data were described below briefly.

4.2.1.1 Plant Height

For this parameter both concentrations of WW proved efficacious, the two gave significantly higher values over GW (Table 23). An increase of 12.06%, 15.74% and 13.98% was obtained under 100%WW application over GW at vegetative, flowering and fruiting stages respectively, followed by 50%WW where the percentage increase was 6.25, 10.34, and 9.22. Among various doses of phosphorus, P_{30} was the optimum dose as it was at par with P_{45} at flowering and fruiting stages. An increase of 40.76%, 50.65% and 38.96% was marked by this dose over P_0 at the three stages studied. Interaction was significant at vegetative stage only where $100\% \times P_{30}$ gave the maximum value by marking an increase of 58.53% over $GW \times P_0$ and it was also at par with $100\% \times P_{45}$. Similarly, the values of $50\% \times P_{30}$ and $GW \times P_{45}$ were also at par. Plant height increased from vegetative to fruiting stage. More percentage increase was observed from vegetative to flowering stage.

Table 23. Effect of GW, 50%WW and 100%WW on plant height (cm) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	25.80	27.60	28.30	27.23	42.00	45.30	49.10	45.47
P_{15}	29.60	32.20	34.40	32.07	51.10	55.20	59.40	55.23
P_{30}	35.90	38.20	40.90	38.33	62.50	70.40	72.60	68.50
P_{45}	38.00	39.40	41.30	39.57	64.20	71.60	73.30	69.70
Mean	32.33	34.35	36.23		54.95	60.63	63.60	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
P_0	57.50	60.10	63.80	60.47				
P_{15}	66.00	71.40	75.60	71.00	Water	0.548	1.620	1.580
P_{30}	77.10	86.00	89.00	84.03	Phosphorus	0.635	1.870	1.830
P_{45}	78.40	87.20	89.60	85.07	Interaction	1.099	NS	NS
Mean	69.75	76.18	79.50					

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

4.2.1.2 Leaf Number Plant^{-1}

It was significantly enhanced under wastewater application over GW (Table 24) as 100%WW registered an increase of 10.86%, 12.61% and 13.85%, whereas an increase of 8.17%, 9.23% and 6.37% was noted under 50%WW. At vegetative stage, both concentrations of wastewater gave at par values. P_{30} was the optimum dose as it was at par with P_{45} at all the stages and recorded an increase of 34.76%, 36.01% and 35.40% over P_0. Contrary to plant height, leaf number increased upto flowering stage only and then it decreased.

4.2.1.3 Leaf Area Plant^{-1}

Significant increase was also seen in the leaf area of the plants irrigated with WW (Table 25) as 100%WW registered an increase of 11.10%, 11.23%, 18.01% over GW, whereas an increase of 7.99%, 7.86% and 14.18% was recorded with 50%WW at the three growth stages. Among fertilizer doses, P_{30} proved optimum as it gave values at par to P_{45} at vegetative and fruiting stages. An increase of 34.78%, 39.60% and 45.40% was recorded over P_0. Among interactions, 100%×P_{30} was the best as it marked an increase of 44.05%, 50.72% and 66.93%, however its value was also at par with 100%×P_{45} and 50%×P_{45} at vegetative and fruiting stages, whereas at flowering at par values were shown by 100%×P_{45} and 100%×P_{30}. Like leaf number, leaf area also increased upto flowering, after which it decreased.

4.2.1.4 Fresh Weight Plant^{-1}

WW increased the fresh weight of the plants (Table 26). Both 100%WW as well as 50%WW performed differently at the three stages studied and gave an increase of 13.00%, 13.47%, 12.07% and 8.05%, 10.69%, 9.44% over GW. Among phosphorus doses, P_{45} gave best results at vegetative and flowering stages, whereas at fruiting stage it showed value at par to P_{30}. The percentage increase with P_{45} was 42.06, 42.50 and 33.96 whereas with P_{30} it was 34.36, 38.82 and 31.37 at the three successive stages of growth. Among interactions 100%×P_{30} gave the optimum value as it was at par with 100%×P_{15} at vegetative and flowering stages and recorded an increase of 50.43%, 53.36% over GW×P_0. Similarly GW×P_{30} was at par with 100%×P_{15} and 50%×P_{15}, also 100%×P_0 was at par with 50%×P_0 and GW×P_{15} at both the stages showing the utility of wastewater in saving phosphatic fertilizer. An

Table 24. Effect of GW, 50%WW and 100%WW on leaf number plant^{-1} of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100% WW	Mean
P_0	14.03	14.88	15.19	14.70	25.33	27.01	27.65	26.66
P_{15}	15.88	16.64	16.97	16.50	28.88	31.00	32.09	30.66
P_{30}	18.14	20.33	20.96	19.81	33.19	37.16	38.43	36.26
P_{45}	19.00	20.65	21.20	20.28	34.41	37.87	39.00	37.09
Mean	16.76	18.13	18.58		30.45	33.26	34.29	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	21.74	22.96	23.43	22.71
P_{15}	24.52	26.24	27.31	26.02
P_{30}	28.40	30.34	33.50	30.75
P_{45}	29.00	30.73	33.80	31.18
Mean	25.92	27.57	29.51	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.664	0.736	0.645
Phosphorus	0.766	0.850	0.745
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 25. Effect of GW, 50%WW and 100%WW on leaf area (cm^2 plant^{-1}) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	283.41	286.10	292.02	287.18	371.18	378.49	396.89	382.19
P_{15}	306.83	325.63	340.13	324.20	413.16	451.44	465.94	443.51
P_{30}	355.44	397.44	408.26	387.05	495.03	546.18	559.44	533.55
P_{45}	362.18	403.16	412.70	392.68	508.26	552.16	566.16	542.19
Mean	326.97	353.08	363.28		446.91	482.07	497.11	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
P_0	309.32	332.18	348.56	330.02				
P_{15}	369.23	410.33	425.23	401.60	Water	5.10	5.097	9.29
P_{30}	423.16	500.06	516.34	479.85	Phosphorus	6.89	5.886	10.73
P_{45}	435.27	512.30	523.63	490.40	Interaction	11.20	10.195	18.58
Mean	384.25	438.72	453.44					

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 26. Effect of GW, 50%WW and 100%WW on plant fresh weight (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	8.11	8.44	8.76	8.44	14.28	15.00	15.55	14.94
P_{15}	8.99	9.77	10.36	9.71	16.00	17.86	18.45	17.44
P_{30}	10.34	11.47	12.20	11.34	18.90	21.43	21.90	20.74
P_{45}	11.32	12.20	12.46	11.99	19.68	21.93	22.25	21.29
Mean	9.69	10.47	10.95		17.22	19.06	19.54	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	23.10	24.36	24.87	24.11
P_{15}	25.48	28.41	29.27	27.72
P_{30}	29.10	32.54	33.37	31.67
P_{45}	30.35	33.00	33.54	32.30
Mean	27.01	29.56	30.27	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.287	0.470	0.569
Phosphorus	0.331	0.542	0.658
Interaction	0.570	0.939	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

increasing trend was observed in the fresh weight of plants from vegetative to fruiting stage.

4.2.1.4 Dry Weight Plant^{-1}

More dry weight got accumulated in the plants irrigated with WW (Table 27). Both 100%WW as well as 50%WW gave an increase of 12.98%, 12.72%, 13.94% and 8.77%, 7.95%, 9.77% over GW. At vegetative stage only both 50%WW and 100%WW were equally effective. P_{30} gave the optimum values at vegetative and flowering stages, whereas at fruiting the value of P_{45} surpassed the value of P_{30}. An increase of 35.32%, 35.63% and 35.36% was shown by P_{30}. Among interactions, 100%×P_{30} proved optimum by marking an increase of 51.56% and 49.58% over GW×P_0 at vegetative and flowering stages respectively. Most other interactions were also at par. Plant dry weight increased with increase in age of the plants, however percentage increase was more from vegetative to flowering stage.

4.2.2 Physiological Parameters

Total chlorophyll content, carotenoids, nitrate reductase activity, carbonic anhydrase activity, leaf nitrogen, phosphorus and potassium contents were studied at vegetative, flowering and fruiting stages.

4.2.2.1 Total Chlorophyll Content

As evident from the table 28 that the total chlorophyll content of leaves irrigated with WW increased over the plants irrigated with GW. The percentage of 10.46, 16.75, 14.21 was registered with 100%WW followed by 50%WW which marked an increase of 2.61%, 10.05% and 9.47%. P_{45} gave the optimum results and recorded an increase of 28.26% and 36.84% at vegetative and flowering stages, however at fruiting values of P_{45} and P_{30} were equal. Interaction was significant only at fruiting where 100%×P_{30} was optimum and it registered an increase of 55.15% over GW×P_0. Like leaf number and leaf area, chlorophyll content increased only upto flowering and then it decreased.

4.2.2.2 Carotenoids

Significant increase was also found in the carotenoid content of plants irrigated with 100% as well as 50%WW (Table 29) while former recorded an increase of 9.02%, 10.87%, 9.88%, with the latter it was 6.97%, 6.46%, 5.86% at the three

Table 27. Effect of GW, 50%WW and 100%WW on plant dry weight (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	2.56	2.70	2.81	2.69	4.76	4.94	5.12	4.94
P_{15}	2.90	3.16	3.26	3.11	5.30	5.80	6.07	5.72
P_{30}	3.35	3.70	3.88	3.64	6.20	6.78	7.12	6.70
P_{45}	3.50	3.82	3.96	3.76	6.36	6.90	7.21	6.82
Mean	3.08	3.35	3.48		5.66	6.11	6.38	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	7.71	8.15	8.32	8.06
P_{15}	8.53	9.40	9.79	9.24
P_{30}	9.93	11.13	11.66	10.91
P_{45}	10.26	11.30	11.76	11.11
Mean	9.11	10.00	10.38	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.200	0.136	0.094
Phosphorus	0.232	0.158	0.154
Interaction	0.401	0.273	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 28. Effect of GW, 50%WW and 100%WW on total chlorophyll content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	1.34	1.38	1.42	1.38	1.81	1.92	1.96	1.90
P_{15}	1.48	1.52	1.60	1.53	1.94	2.21	2.30	2.15
P_{30}	1.60	1.64	1.84	1.69	2.21	2.44	2.70	2.45
P_{45}	1.68	1.72	1.90	1.77	2.40	2.62	2.79	2.60
Mean	1.53	1.57	1.69		2.09	2.30	2.44	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	1.65	1.71	1.76	1.71
P_{15}	1.82	1.88	1.95	1.88
P_{30}	2.00	2.33	2.56	2.30
P_{45}	2.13	2.40	2.39	2.31
Mean	1.90	2.08	2.17	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.047	0.141	0.063
Phosphorus	0.054	0.131	0.072
Interaction	NS	NS	0.013

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

successive stages. At vegetative stage both the concentrations of wastewater were at par. P_{30} proved optimum dose and an increase of 27.79%, 29.26% and 23.76% was obtained with it. Like total chlorophyll, carotenoids also increased upto flowering and then decreased.

4.2.2.3 Nitrate Reductase Activity (NRA)

Both concentrations of wastewater performed well to enhance this enzymatic activity and differed significantly from ground water at all the stages of growth (Table 30). An increase of 11.51%, 12.32%, 11.54% by 100%WW was recorded over GW, whereas 50%WW recorded an increase of 7.86%, 9.36% and 7.39%. Fertilizer dose, P_{30} proved optimum as it was at par with P_{45} at all the stages. Interaction was non significant. This enzymatic activity also increased upto flowering after which it decreased.

4.2.2.4 Carbonic Anhydrase (CA) Activity

For this enzyme also wastewater proved effective, and a significant increase was found over GW (Table 31). At vegetative and flowering stages, 100%WW and 50%WW were at par and former recorded an increase of 8.83%, 9.62%, but at fruiting both concentrations performed differently and recorded an increase of 10.05% and 5.82% respectively. P_{30} was optimum dose as it gave value at par to P_{45} and recorded an increase of 35.55%, 30.38% and 33.94% over P_0. Like NRA, CA also decreased after flowering.

4.2.2.5 Leaf Nitrogen Content

WW increased the nitrogen content of leaves over GW (Table 32). Both concentrations performed differently at flowering and fruiting stages. 100%WW recorded an increase of 8.24%, and , 11.71%, however an increase of 4.90% and 7.81% was shown by 50%WW. At vegetative stage their response was similar. P_{45} gave optimum value and recorded an increase of 32.04%, 29.77% and 34.69%. It may be observed that nitrogen uptake was enhanced with phosphorus application showing the synergistic effect between the two nutrients.

4.2.26 Leaf Phosphorus Content

A significant increase was also observed in phosphorus content of the plants irrigated with wastewater (Table 33). 100%WW as well as 50%WW performed

Table 29. Effect of GW, 50%WW and 100%WW on carotenoid content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	0.424	0.441	0.452	0.439	0.532	0.570	0.590	0.564
P_{15}	0.463	0.498	0.512	0.491	0.614	0.657	0.681	0.651
P_{30}	0.528	0.571	0.584	0.561	0.690	0.733	0.764	0.729
P_{45}	0.536	0.576	0.580	0.564	0.702	0.745	0.780	0.742
Mean	0.488	0.522	0.532		0.635	0.676	0.704	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	0.510	0.548	0.570	0.543
P_{15}	0.584	0.617	0.631	0.611
P_{30}	0.640	0.673	0.704	0.672
P_{45}	0.652	0.685	0.720	0.686
Mean	0.597	0.632	0.656	

CD at 5%			
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.017	0.017	0.017
Phosphorus	0.019	0.020	0.020
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 30. Effect of GW, 50%WW and 100%WW on nitrate reductase activity (μ mol g^{-1} leaf fresh weight h^{-1}) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	287.18	301.31	312.97	300.49	342.52	364.46	377.45	361.48
P_{15}	307.54	332.23	358.40	332.72	389.15	410.89	428.84	409.63
P_{30}	371.23	416.18	420.20	402.54	432.49	489.38	500.23	474.03
P_{45}	384.26	406.56	414.11	401.64	441.68	491.52	497.18	476.79
Mean	337.55	364.07	376.42		401.46	439.06	450.93	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	316.72	331.16	344.45	330.78
P_{15}	351.68	378.44	397.90	376.01
P_{30}	410.13	446.61	460.40	439.05
P_{45}	418.70	451.74	467.31	445.92
Mean	374.31	401.99	417.52	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	9.45	10.27	10.53
Phosphorus	10.91	11.88	12.16
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 31. Effect of GW, 50%WW and 100%WW on carbonic anhydrase activity [mol (CO_2) kg^{-1} (leaf fresh mass) s^{-1}] of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	2.58	2.72	2.80	2.70	3.70	3.87	3.98	3.85
P_{15}	2.97	3.20	3.30	3.16	4.07	4.35	4.56	4.33
P_{30}	3.48	3.70	3.81	3.66	4.58	5.37	5.10	5.02
P_{45}	3.64	3.82	3.90	3.79	4.67	5.26	5.05	4.99
Mean	3.17	3.36	3.45		4.26	4.71	4.67	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	3.14	3.26	3.42	3.27
P_{15}	3.59	3.88	4.08	3.85
P_{30}	4.11	4.40	4.62	4.38
P_{45}	4.29	4.46	4.50	4.42
Mean	3.78	4.00	4.16	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.061	0.069	0.030
Phosphorus	0.072	0.080	0.173
Interaction	NS	0.139	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 32. Effect of GW, 50%WW and 100%WW on leaf nitrogen content (%) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	3.25	3.34	3.43	3.34	2.98	3.09	3.20	3.09
P_{15}	3.52	3.74	3.84	3.70	3.39	3.48	3.64	3.50
P_{30}	4.00	4.43	4.56	4.33	3.71	3.92	4.08	3.90
P_{45}	4.28	4.49	4.45	4.41	3.80	4.05	4.18	4.01
Mean	3.76	4.00	4.07		3.47	3.64	3.78	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	2.79	2.96	3.08	2.94
P_{15}	3.26	3.48	3.60	3.45
P_{30}	3.55	3.90	4.00	3.82
P_{45}	3.72	4.02	4.13	3.96
Mean	3.33	3.59	3.72	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.085	0.086	0.112
Phosphorus	0.098	0.097	0.129
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

differently at vegetative and flowering stages, whereas at fruiting both were at par. The percentage increase of 12.29, 10.98, 10.83 and 10.06, 7.58, 8.49 was observed at three successive stages of growth. Phosphorus application enhanced the uptake upto P_{45} level, which was the best dose as it recorded an increase of 39.66%, 40.47% and 34.86%. Among various interactions, 100%×P_{30} proved optimum and recorded an increase of 52.68% and 50.73% over GW×P_0 at vegetative and flowering stages. At both stages 100%×P_{45}, 100%×P_{30} and 50%×P_{45} gave at par values. Like nitrogen content, phosphorus content also decreased with increase in age of the plants.

4.2.2.7 Leaf Potassium Content

Table 34 showed that with wastewater application significant increase was observed in potassium content. Thus an increase of 11.67%, 12.92%, 10.98% and 6.37%, 7.87%, 6.65% was shown by 100%WW and 50%WW at three successive stages of growth. Like N and P, K uptake was also enhanced by phosphorus application. P_{30} gave optimum values and registered an increase of 30.65%, 32.70% and 31.48% at the three stages studied. Among interactions, 100%×P_{30} gave the best results and was at par with 100%×P_{45} at vegetative and flowering stages. An increase of 44.31%, 49.33% and 48.97% was recorded by this treatment. Among other interactions GW×P_{15} gave value at par to 100%×P_0. Leaf potassium content also decreased with increase in plant age. Among the three nutrients, like experiment I, here also K contents were maximum followed by N and P.

4.2.3 Yield and Quality Parameters

Yield and quality parameters like siliqua length, number of siliqua plant^{-1}, seeds siliqua^{-1}, 1000 seed weight, seed yield plant^{-1}, plant biomass, oil content, oil yield, seed protein, and heavy metal contents of seeds were observed at harvest.

4.2.3.1 Siliqua Length

Wastewater proved beneficial in increasing the length of siliqua (Table 35). 50%WW was at par with 100%WW as well as with GW. An increase of 8.08% and 4.62% was shown by 100%WW and 50%WW respectively. Among various doses of phosphorus, P_{30} proved optimum as it recorded an increase of 24.59% over control and was at par with P_{45}.

Table 33. Effect of GW, 50%WW and 100%WW on leaf phosphorus content (%) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	0.448	0.470	0.488	0.469	0.412	0.430	0.447	0.430
P_{15}	0.509	0.548	0.566	0.541	0.475	0.508	0.527	0.503
P_{30}	0.582	0.664	0.684	0.643	0.550	0.600	0.621	0.590
P_{45}	0.610	0.680	0.675	0.655	0.567	0.617	0.628	0.604
Mean	0.537	0.591	0.603		0.501	0.539	0.556	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	0.400	0.417	0.432	0.416
P_{15}	0.448	0.471	0.490	0.470
P_{30}	0.513	0.576	0.594	0.561
P_{45}	0.524	0.580	0.572	0.559
Mean	0.471	0.511	0.522	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.006	0.008	0.013
Phosphorus	0.007	0.009	0.015
Interaction	0.012	0.016	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 34. Effect of GW, 50%WW and 100%WW on leaf potassium content (%) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	3.25	3.36	3.48	3.36	3.00	3.18	3.28	3.15
P_{15}	3.57	3.71	3.89	3.72	3.40	3.66	3.81	3.62
P_{30}	4.03	4.45	4.69	4.39	3.85	4.21	4.48	4.18
P_{45}	4.21	4.51	4.78	4.50	4.00	4.30	4.50	4.27
Mean	3.77	4.01	4.21		3.56	3.84	4.02	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
P_0	2.92	3.10	3.21	3.08
P_{15}	3.32	3.50	3.67	3.50
P_{30}	3.74	4.06	4.35	4.05
P_{45}	3.86	4.11	4.11	4.03
Mean	3.46	3.69	3.84	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.072	0.066	0.066
Phosphorus	0.083	0.096	0.093
Interaction	0.144	0.141	0.147

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

4.2.3.2 Siliqua Number Plant^{-1}

Significantly higher number of siliquae were produced by plants irrigated with city wastewater (Table 35) thus 100%WW recorded an increase of 9.22% over GW, whereas an increase of 6.28% was marked with 50%WW. P_{30} being at par with P_{45} proved optimum recorded an increase of 24.51% over P_0. Among various interactions, 100%×P_{30} gave maximum value and registered an increase of 35.52% over GW×P_0, however it was at par with 100%×P_{45}. Further 100%×P_{15} was also at par with GW×P_{30} while it was at par with 50%×P_{15}. It was also observed that 100%×P_0 was at par with GW×P_{15} showing the utility of wastewater as a source of phosphorus.

4.2.3.3 Seeds Siliqua^{-1}

Wastewater application enhanced the number of seeds per siliqua (Table 35). An increase of 4.54% and 2.96% was shown by 100%WW and 50%WW respectively. P_{45} gave maximum value as it recorded an increase of 12.86% over control. Among interactions 100%×P_{30} gave the maximum value and marked an increase of 16.95% over GW×P_0.

4.2.3.4 1000 Seed Weight

Seeds of plants irrigated with WW attained more weight over the seeds of plants irrigated with GW (Table 36). 3.76 and 2.32 was the percentage increase observed in the seed weight of the plants irrigated with 100%WW and 50%WW over GW. More 1000 seed weight was obtained with the higher dose of phosphorus, thus P_{45} proved the best and registered an increase of 10.59% over P_0. Among interactions, 100%×P_{45} proved best and recorded an increase of 14.83% over GW×P_0. All other interactions were different except 100%×P_{30} and 50%×P_{45} which were at par.

4.2.3.5 Seed Yield Plant^{-1}

Higher seed yield was attained by the plants irrigated with city wastewater (Table 36). Increase in terms of seed yield was 14.07% with 100%WW, whereas with 50%WW it was 9.26% over GW. Fertilizer dose P_{30} gave the optimum value, as it recorded an increase of 33.61% over P_0 and was at par with P_{45} which was at luxury consumption highlighting the wasteful consumption of this extra dose. It may be also pointed out that P_{15} proved deficient. Among various interactions, 100%×P_{30} was optimum; it recorded an increase of 48.21% over GW × P_0 and was at par with

Table 35. Effect of GW, 50%WW and 100%WW on siliqua length (cm), siliqua number plant^{-1} and seeds siliqua^{-1} of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Siliqua length				Siliqua number			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	5.35	5.42	5.58	5.45	94.16	97.28	99.32	96.92
P_{15}	5.65	6.25	6.41	6.10	100.86	108.04	110.38	106.43
P_{30}	6.52	6.79	7.06	6.79	112.54	121.89	127.61	120.68
P_{45}	6.72	6.90	7.14	6.92	116.19	123.15	125.53	121.62
Mean	6.06	6.34	6.55		105.94	112.59	115.71	

	Seeds siliqua^{-1}			
	GW	50%WW	100%WW	Mean
P_0	13.57	13.85	14.09	13.84
P_{15}	14.25	14.70	14.96	14.64
P_{30}	15.08	15.64	15.95	15.56
P_{45}	15.26	15.70	15.89	15.62
Mean	14.54	14.97	15.22	

CD at 5%	Siliqua length	Siliqua number	Seeds siliqua^{-1}
Water	0.302	1.524	0.071
Phosphorus	0.348	1.759	0.082
Interaction	NS	3.047	0.141

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

Table 36. Effect of GW, 50%WW and 100%WW on 1000-seed weight (g), seed yield (g $plant^{-1}$) and biomass (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	1000-seed weight				Seed yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	5.26	5.39	5.48	5.38	6.99	7.20	7.50	7.23
P_{15}	5.52	5.60	5.69	5.60	7.67	8.35	8.70	8.24
P_{30}	5.75	5.90	6.00	5.88	8.79	9.83	10.36	9.66
P_{45}	5.82	6.00	6.04	5.95	8.98	10.01	10.40	9.80
Mean	5.59	5.72	5.80		8.10	8.85	9.24	

	Biomass			
	GW	50%WW	100%WW	Mean
P_0	11.42	12.28	12.53	12.08
P_{15}	13.13	14.31	14.78	14.07
P_{30}	15.06	15.84	16.20	15.70
P_{45}	15.31	16.00	16.32	15.88
Mean	13.73	14.61	14.96	

CD at 5%	1000-seed weight	Seed yield	Biomass
Water	0.020	0.153	0.202
Phosphorus	0.023	0.177	0.233
Interaction	0.039	0.307	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

$100\% \times P_{45}$. Further $GW \times P_{45}$, $GW \times P_{30}$ and $100\% \times P_{15}$ gave at par values. Again $100\% \times P_0$ was at par with $50\% \times P_0$, it showed values at par to $GW \times P_{15}$ proving the utility of wastewater.

4.2.3.6 Plant Biomass

Biomass of plants irrigated with WW was increased over the plants irrigated with GW (Table 36). Both 100%WW and 50%WW performed differently and marked an increase of 8.96% and 6.41% over GW. Among various doses of phosphorus, P_{30} gave the maximum value being at par with P_{45} and recorded an increase of 29.97% over P_0 Interactions were non significant.

4.2.3.7 Oil Content

Significant increase was observed in the oil content of plants under WW (Table 37). Both 100%WW and 50%WW proved equally effective and the former recorded an increase of 3.68% over GW. Fertilizer doses and their interactions proved non significant for increasing the oil content of the seeds.

4.2.3.8 Oil Yield

Irrigation with city wastewater increased the oil yield of the plants (Table 37). 100%WW and 50%WW performed differently and recorded an increase of 18.13% and 12.87% over GW. P_{30} was optimum as it was at par with P_{45} and recorded an increase of 36.93% over P_0. Among interactions, $100\% \times P_{30}$ recorded an increase of 56.51% over $GW \times P_0$, however its value was at par to $100\% \times P_{45}$. In addition at par values were also shown by $100\% \times P_{15}$ and $GW \times P_{45}$. Similarly $100\% \times P_0$ and $GW \times P_{15}$ were equally effective.

4.2.3.9 Seed Protein Content

Non significant effect was shown by WW as well as fertilizer in this parameter (Table 37).

4.3 EXPERIMENT -III

The aim of this pot experiment, conducted on the same variety of *Brassica juncea*, was to study the comparative effect of wastewater and ground water under four doses of potassium. All the growth, physiological, yield and quality parameters studied earlier were observed in this experiment also.

Table 37. Effect of GW, 50%WW and 100%WW on oil content (%), oil yield (g plant^{-1}) and seed protein content (%) of *Brassica juncea* cv. Varuna with different levels of phosphorus.

Treatments	Oil content				Oil yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
P_0	41.71	42.34	42.95	42.33	2.92	3.05	3.22	3.06
P_{15}	42.05	44.28	44.32	43.55	3.23	3.70	3.86	3.60
P_{30}	42.25	43.62	44.13	43.33	3.71	4.29	4.57	4.19
P_{45}	42.63	44.02	43.45	43.36	3.83	4.41	4.52	4.25
Mean	42.16	43.57	43.71		3.42	3.86	4.04	

	Seed protein content			
	GW	50%WW	100%WW	Mean
P_0	21.30	22.30	22.70	22.10
P_{15}	22.00	22.60	23.10	22.57
P_{30}	22.70	23.80	24.10	23.53
P_{45}	23.40	24.00	24.90	24.10
Mean	22.35	23.18	23.70	

CD at 5%	Oil content	Oil yield	Seed protein content
Water	1.657	0.058	NS
Phosphorus	NS	0.079	NS
Interaction	NS	0.123	NS

N.B.: Subscript values denote the amount of phosphorus (P) in kg ha^{-1}. A uniform dose of N_{40} and K_{20} in kg ha^{-1} was also applied basally.

4.3.1 Growth Parameters

Plant height, leaf number leaf area, plant fresh weight and plant dry weight were observed at three stages. The significant data were briefly described below.

4.3.1.1 Plant Height

Wastewater produced lengthier plants in comparison with GW (Table 38). 100%WW showed an increase of 12.35%, 13.36% and 12.18% at vegetative, flowering and fruiting stages respectively, followed by 50%WW. Fertilizer dose K_{30} was the best as it gave maximum values for this parameter being critically different from other doses. It recorded an increase of 34.89%, 43.33% and 38.27% over K_0. Interaction was significant only at fruiting stage, where 100%×K_{30} gave maximum value by marking an increase of 51.68% over GW×K_0, its value was at par to 50%×K_{30}. Further at par values were given by GW×K_{20}, 50%×K_{10} and 100%×K_{10}. Plant height increased from vegetative to fruiting stage, however percentage increase was more from vegetative to flowering stage in comparison from flowering to fruiting stage.

4.3.1.2 Leaf Number Plant^{-1}

WW resulted in the production of more leaves in mustard over GW (Table 39). An increase of 13.63%, 15.72%, 18.12% and 9.65%, 13.63%, 10.07% was registered with 100%WW and 50%WW respectively at the three stages K_{20} was at par with K_{30} at flowering and fruiting whereas at vegetative stage K_{30} gave maximum value. An increase of 34.15%, 41.63% and 37.33% was shown by K_{20} over K_0. Interaction was non significant at all the stages. Leaf number increased only upto flowering stage and then it decreased.

4.3.1.3 Leaf Area Plant^{-1}

As evident from the table 40 that plants irrigated with WW attained more leaf area. Both concentrations of WW performed differently at three stages studied. An increase of 8.27%, 10.73% and 13.85% was noted with 100%WW over GW. K_{20} proved optimum dose by giving maximum values at vegetative and flowering stages, but at fruiting stage K_{30} gave maximum value. An increase of 30.40%, 40.04% and 40.72% was noted with former treatment. Among interactions, 100%×K_{30} was optimum at fruiting and it recorded an increase of 61.83% over GW×K_0. At par values

Table 38. Effect of GW, 50%WW and 100%WW on plant height (cm) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	26.30	27.80	28.70	27.60	45.00	48.90	51.20	48.37
K_{10}	29.40	31.00	32.60	31.00	53.40	59.20	61.80	58.13
K_{20}	33.22	36.30	38.00	35.84	62.60	68.50	71.90	67.67
K_{30}	34.80	37.20	39.70	37.23	66.00	69.60	72.40	69.33
Mean	30.93	33.08	34.75		56.75	61.55	64.33	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
K_0	62.30	66.00	67.90	65.40				
K_{10}	70.40	79.10	78.20	75.90	Water	1.16	0.889	1.292
K_{20}	80.00	90.30	91.80	87.37	Potassium	1.34	1.029	1.493
K_{30}	83.60	93.20	94.50	90.43	Interaction	NS	NS	2.585
Mean	74.08	82.15	83.10					

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 39. Effect of GW, 50%WW and 100%WW on leaf number plant^{-1} of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	13.74	14.27	14.60	14.20	25.03	26.74	27.08	26.28
K_{10}	14.20	16.18	16.94	15.77	28.49	32.00	32.80	31.10
K_{20}	17.35	19.51	20.30	19.05	33.16	38.85	39.65	37.22
K_{30}	19.00	20.52	21.21	20.24	33.94	39.48	40.08	37.83
Mean	16.07	17.62	18.26		30.16	34.27	34.90	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	21.68	23.00	23.63	22.77
K_{10}	23.59	25.64	26.70	25.31
K_{20}	27.80	31.32	34.70	31.27
K_{30}	28.64	32.00	35.14	31.93
Mean	25.43	27.99	30.04	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.545	0.529	0.89
Potassium	0.630	0.611	1.03
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 40. Effect of GW, 50%WW and 100%WW on leaf area (cm^2 $plant^{-1}$) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	284.16	298.34	304.90	295.80	371.44	388.23	401.53	387.07
K_{10}	316.57	352.26	359.17	342.67	428.77	479.06	493.51	467.11
K_{20}	367.72	389.41	400.13	385.75	506.81	557.24	562.12	542.06
K_{30}	382.89	396.62	398.84	392.78	518.21	564.28	564.00	548.83
Mean	337.84	359.16	365.76		456.31	497.20	505.29	

Treatments	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	321.37	348.16	357.40	342.31
K_{10}	375.46	418.04	436.25	409.92
K_{20}	442.42	494.31	508.38	481.70
K_{30}	461.28	510.23	520.08	497.20
Mean	400.13	442.69	455.53	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	6.56	5.95	4.65
Potassium	7.53	6.84	5.36
Interaction	NS	11.50	9.29

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

were shown by 50%×K_{30} and 100%×K_{20}, but at flowering 100%×K_{20} gave the optimum value being at par with 100%×K_{30}, 50%×K_{30}, 50%×K_{20}. Like leaf number, leaf area also increased upto flowering stage and then decreased.

4.3.1.4 Fresh weight Plant^{-1}

Plants irrigated with WW attained more fresh weight over the plants irrigated with GW (Table 41). An increase of 11.75%, 14.27%, 10.81% was shown by 100%WW, whereas 50%WW showed an increase of 8.44%, 11.46% and 7.44% over GW. Among various doses of potassium, K_{20} was the optimum, as it was at par with K_{30} at flowering and fruiting whereas at vegetative K_{30} was comparatively better. Interaction was significant at flowering stage only where 100%×K_{20} gave maximum value by recording an increase of 51.91% over GW×K_0. Among other interactions GW×K_{20} was at par with GW×K_{30} and it was also at par with 100%×K_{10}. Unlike leaf number and leaf area, plant fresh weight increased with the increase in the age of the plants. However, more percentage increase was found from vegetative to flowering stage.

4.3.1.5 Dry Weight Plant^{-1}

Plants irrigated with WW accumulated more dry weight over GW (Table 42). Both concentrations of wastewater performed differently. K_{30} proved optimum as it gave values different from K_{20} at vegetative and fruiting stages whereas at flowering stage K_{20} and K_{30} gave at par values. An increase of 43.30%, 37.09% and 36.90% was recorded with the optimum dose. Interaction was significant only at fruiting where 100%×K_{20} gave maximum value by recording an increase of 49.21% over GW×K_0. Most other interactions gave at par values. Like fresh weight, plant dry weight also increased with the increase in the age of plants. However, increase was more from vegetative to flowering stage.

4.3.2 Physiological Parameters

Total chlorophyll content, carotenoids, nitrate reductase activity, carbonic anhydrase activity, leaf nitrogen, phosphorus and potassium contents were studied at vegetative, flowering and fruiting stages. Data were briefly described below.

Table 41. Effect of GW, 50%WW and 100%WW on plant fresh weight (g plant^{-1}) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	8.04	8.50	8.73	8.42	14.16	14.94	15.33	14.81
K_{10}	9.08	9.88	10.25	9.74	15.78	17.30	17.91	17.00
K_{20}	10.51	11.63	12.08	11.41	18.25	21.00	21.51	20.25
K_{30}	11.16	12.10	12.30	11.85	18.80	21.42	21.81	20.68
Mean	9.70	10.53	10.84		16.75	18.67	19.14	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	22.81	24.41	25.04	24.09
K_{10}	25.52	28.00	29.06	27.53
K_{20}	29.56	31.75	32.70	31.34
K_{30}	30.24	32.00	32.98	31.74
Mean	27.03	29.04	29.95	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.365	0.464	0.726
Potassium	0.425	0.533	0.828
Interaction	NS	0.927	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 42. Effect of GW, 50%WW and 100%WW on plant dry weight (g plant^{-1}) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	2.50	2.63	2.71	2.61	4.68	4.90	5.06	4.88
K_{10}	2.83	3.10	3.24	3.06	5.21	5.66	5.91	5.59
K_{20}	3.40	3.60	3.70	3.57	6.15	6.60	6.94	6.56
K_{30}	3.63	3.75	3.83	3.74	6.30	6.72	7.05	6.69
Mean	3.09	3.27	3.37		5.59	5.97	6.24	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	7.64	7.91	8.28	7.94
K_{10}	8.43	9.34	9.62	9.13
K_{20}	9.75	11.00	11.40	10.72
K_{30}	9.94	11.13	11.53	10.87
Mean	8.94	9.85	10.21	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.097	0.259	0.121
Potassium	0.158	0.299	0.141
Interaction	NS	NS	0.244

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

4.3.2.1 Total Chlorophyll Content

Wastewater increased the total chlorophyll content of the plants (Table 43). At vegetative stage both concentrations were equally effective however at flowering and fruiting the two performed differently. An increase of 10.20%, 13.73%, 13.23% and 6.12%, 8.82%, 5.82% was marked with 100%WW and 50%WW. K_{20} gave the optimum value being at par with higher potassium dose K_{30}. Optimum dose registered an increase of 33.86%, 43.60% and 39.13% over K_0. Interaction was non significant. Chlorophyll content increased upto flowering.

4.3.2.2 Carotenoids

Like chlorophyll content, wastewater also enhanced the carotenoid content of leaves (Table 44). 100%WW was more effective and recorded an increase of 6.09%, 8.37% and 7.79% over GW, followed by 50%WW. Among various doses K_{30} gave maximum values at three successive stages and marked an increase of 29.60%, 32.18% and 32.45% over K_0. Carotenoids followed the same trend as observed in total chlorophyll content.

4.3.2.3 Nitrate Reductase Activity (NRA)

Nitrate reductase activity got enhanced by the application of WW (Table 45) 100%WW recorded an increase of 12.22%, 11.54%, 11.75% whereas an increase of 7.11%, 9.50% and 7.79% was shown by 50%WW. K_{20} was the optimum dose being at par with K_{30} at all the stages. The former marked an increase of 33.85%, 35.96% and 37.15% over K_0. Interaction was non significant. NRA increased from vegetative to flowering stage and then decreased.

4.3.2.4 Carbonic Anhydrase (CA) Activity

It may be noted from the table 46 that an increase in enzymatic activity was observed in the plants irrigated with WW. An increase of 6.63%, 9.29% and 14.00% was recorded with 100%WW whereas 50%WW marked an increase of 4.52%, 5.24%, 8.86% over GW. K_{20} was the optimum dose as it was at par with K_{30} and registered an increase 25.76%, 26.60% and 25.15% over K_0. Interaction was non significant like NRA. CA also increased from vegetative to flowering stage and then it decreased.

Table 43. Effect of GW, 50%WW and 100%WW on total chlorophyll content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	1.21	1.27	1.33	1.27	1.62	1.73	1.80	1.72
K_{10}	1.36	1.46	1.53	1.45	1.89	2.08	2.18	2.05
K_{20}	1.61	1.72	1.78	1.70	2.27	2.52	2.62	2.47
K_{30}	1.68	1.78	1.82	1.76	2.36	2.56	2.68	2.53
Mean	1.47	1.56	1.62		2.04	2.22	2.32	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	1.54	1.60	1.69	1.61
K_{10}	1.75	1.87	2.00	1.87
K_{20}	2.09	2.24	2.40	2.24
K_{30}	2.18	2.30	2.45	2.31
Mean	1.89	2.00	2.14	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.076	0.066	0.086
Potassium	0.088	0.075	0.097
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 44. Effect of GW, 50%WW and 100%WW on carotenoid content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	0.423	0.450	0.464	0.446	0.552	0.580	0.601	0.578
K_{10}	0.489	0.521	0.538	0.516	0.623	0.664	0.694	0.660
K_{20}	0.550	0.563	0.575	0.563	0.713	0.750	0.770	0.744
K_{30}	0.574	0.575	0.584	0.578	0.740	0.768	0.783	0.764
Mean	0.509	0.527	0.540		0.657	0.691	0.712	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	0.503	0.529	0.548	0.527
K_{10}	0.566	0.601	0.639	0.602
K_{20}	0.654	0.668	0.695	0.672
K_{30}	0.684	0.692	0.718	0.698
Mean	0.602	0.623	0.650	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.009	0.016	0.019
Potassium	0.011	0.018	0.023
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 45. Effect of GW, 50%WW and 100%WW on nitrate reductase activity (μ mol g^{-1} leaf fresh weight h^{-1}) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	291.13	300.05	316.75	302.64	334.16	352.81	366.74	351.24
K_{10}	328.53	350.86	369.92	349.77	380.21	419.34	426.98	408.84
K_{20}	375.64	410.35	429.22	405.07	440.77	491.62	500.24	477.54
K_{30}	387.28	419.64	435.62	414.18	456.14	500.64	503.20	486.66
Mean	345.65	370.23	387.88		402.82	441.10	449.29	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	306.81	328.34	337.49	324.21
K_{10}	352.44	381.23	397.55	377.07
K_{20}	415.28	450.32	468.34	444.65
K_{30}	428.11	459.83	475.81	454.58
Mean	375.66	404.93	419.80	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	8.35	8.48	9.74
Potassium	9.65	9.79	11.25
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 46. Effect of GW, 50%WW and 100%WW on carbonic anhydrase activity [mol (CO_2) kg^{-1} (leaf fresh mass) s^{-1}] of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	2.86	2.95	3.04	2.95	3.62	3.76	3.89	3.76
K_{10}	3.20	3.38	3.49	3.36	3.96	4.28	4.47	4.24
K_{20}	3.58	3.75	3.80	3.71	4.54	4.78	4.96	4.76
K_{30}	3.63	3.80	3.84	3.76	4.68	4.86	5.03	4.86
Mean	3.32	3.47	3.54		4.20	4.42	4.59	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	3.22	3.36	3.44	3.34
K_{10}	3.32	3.50	3.78	3.53
K_{20}	3.80	4.26	4.48	4.18
K_{30}	3.93	4.38	4.57	4.29
Mean	3.57	3.88	4.07	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.158	0.145	0.161
Potassium	0.182	0.167	0.185
Interaction	NS	0.290	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

4.3.2.5 Leaf Nitrogen Content

It increased with the wastewater application (Table 47). The increase with 100%WW was 9.52%, 9.33% and 6.98% over GW at vegetative, flowering and fruiting stages. Among potassium treatments, K_{20} proved optimum and recorded an increase of 28.96%, 30.59% and 33.68% over K_0. Interaction was significant at fruiting only. 100%×K_{20} was optimum by recording an increase of 43.16% over GW×K_0, its value was at par with 100%×K_{30}, 50%×K_{30} and 50%×K_{20}. Nitrogen content of leaves decreased with age.

4.3.2.6 Leaf Phosphorus Content

WW also increased the phosphorus content of leaves. As evident from the table 48 that 100%WW recorded an increase of 10.90%, 11.74%, 10.84% at vegetative, flowering and fruiting stages respectively over GW. For this parameter K_{30} gave maximum values at vegetative and fruiting stages, showing synergistic relationship between the two nutrients. An increase of 38.28%, 40.32% and 39.95% was recorded by the optimum dose. Among interactions, 100%×K_{20} gave the optimum values by recording an increase of 48.47% and 51.87%, however its values were at par with 100%×K_{30} and 50%×K_{30}. Rest of the values were critically different. Leaf phosphorus content also decreased with increase in age of the plants.

4.3.2.6 Leaf Potassium Content

As evident from the table 49, WW also enhanced potassium, thus an increase of 8.81%, 13.35%, 11.17% and 4.15%, 9.38%, 7.74% was marked with 100%WW and 50%WW respectively. At all the stages of growth K_{30} proved beneficial and recorded an increase of 33.14%, 35.24% and 37.38% showing the positive co-relation. Interaction was significant at flowering only where 100%×K_{20} gave maximum value by marking an increase of 49.49% over GW×K_0 and its value was at par to 100%×K_{30}, 50%×K_{30}. Also the values of 100%×K_0, GW×K_{10} were at par. A decreasing trend like nitrogen and phosphorus contents was observed with increase in age of the plants.

4.3.3 Yield and Quality Parameters

Siliqua length, number of siliqua plant^{-1}, seeds siliqua^{-1}, 1000 seed weight, seed yield plant^{-1}, biomass plant^{-1}, oil content, oil yield, seed protein content and

Table 47. Effect of GW, 50%WW and 100%WW on leaf nitrogen content (%) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	3.25	3.34	3.46	3.35	2.91	3.05	3.15	3.04
K_{10}	3.60	3.80	4.03	3.81	3.21	3.48	3.61	3.43
K_{20}	4.00	4.44	4.51	4.32	3.75	4.00	4.17	3.97
K_{30}	4.26	4.56	4.56	4.46	3.83	4.04	4.06	3.98
Mean	3.78	4.04	4.14		3.43	3.64	3.75	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	2.78	2.92	3.03	2.91
K_{10}	3.29	3.56	3.70	3.52
K_{20}	3.77	3.91	3.98	3.89
K_{30}	3.90	3.99	4.01	3.97
Mean	3.44	3.60	3.68	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.169	0.062	0.069
Potassium	0.195	0.072	0.080
Interaction	NS	NS	0.095

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 48. Effect of GW, 50%WW and 100%WW on leaf phosphorus content (%) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	0.458	0.480	0.496	0.478	0.420	0.440	0.457	0.439
K_{10}	0.513	0.564	0.584	0.554	0.483	0.536	0.550	0.523
K_{20}	0.604	0.667	0.680	0.650	0.564	0.620	0.635	0.606
K_{30}	0.623	0.680	0.680	0.661	0.578	0.629	0.641	0.616
Mean	0.550	0.598	0.610		0.511	0.556	0.571	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	0.401	0.420	0.432	0.418
K_{10}	0.465	0.506	0.524	0.498
K_{20}	0.537	0.582	0.609	0.576
K_{30}	0.554	0.598	0.603	0.585
Mean	0.489	0.527	0.542	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.006	0.009	0.007
Potassium	0.007	0.011	0.008
Interaction	0.012	NS	0.013

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 49. Effect of GW, 50%WW and 100%WW on leaf potassium content (%) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	3.22	3.42	3.50	3.38	2.95	3.13	3.36	3.15
K_{10}	3.65	3.80	4.08	3.84	3.25	3.62	3.75	3.54
K_{20}	4.20	4.36	4.58	4.38	3.89	4.29	4.41	4.20
K_{30}	4.36	4.50	4.64	4.50	4.00	4.36	4.42	4.26
Mean	3.86	4.02	4.20		3.52	3.85	3.99	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
K_0	2.89	3.08	3.17	3.05
K_{10}	3.25	3.51	3.67	3.48
K_{20}	3.84	4.19	4.32	4.12
K_{30}	3.96	4.24	4.36	4.19
Mean	3.49	3.76	3.88	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.042	0.036	0.045
Potassium	0.046	0.043	0.049
Interaction	NS	0.078	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

heavy metal contents of seeds were observed at the time of harvest. The significant data were discussed below.

4.3.3.1 Siliqua length

As evident from the table 50 that for increasing the length of siliqua, effect of wastewater proved non significant. However, among various doses of potassium K_{30} gave the optimum value being critically different from K_{20} and recorded an increase of 31.43% over K_0.

4.3.3.2 Siliqua Number Plant^{-1}

City wastewater increased this parameter also over the plants irrigated with GW (Table 50). Among potassium doses K_{20} was optimum as it gave value at par to K_{30}. An increase of 22.86% was shown by the former dose showing the luxury consumption of potassium by the latter, whereas K_{10}, although recorded an increase of 10.95% proved deficient.

4.3.3.3 Seeds Siliqua^{-1}

WW proved beneficial as it enhanced seed number by 4.18% and 2.67% with 100%WW and 50%WW respectively over GW (Table 50). Among various doses of potassium, K_{20} proved optimum by marking an increase of 11.21% and its value was at par to K_{30} while K_{10} proved deficient dose.

4.3.3.4 1000 Seed Weight

WW produced heavier seeds than GW (Table 51). Both concentrations increased 1000 seed weight and performed differently. The former recorded an increase of 5.19% whereas an increase of 3.90% was shown by the latter. K_{20} proved optimum as it was at par with K_{30} and recorded an increase of 11.26% over K_0.

4.3.3.5 Seed Yield Plant^{-1}

Along with growth and yield parameters wastewater also enhanced the seed yield of the plants and the two concentrations performed differently (Table 51). 100%WW gave an increase of 13.23%, whereas an increase of 8.41% was shown by 50%WW. Among various doses of potassium, K_{20} gave the value at par to K_{30}, thus proved optimum dose and recorded an increase of 32.04% over K_0 while K_{30} was wasteful dose as it could not enhance the seed yield, whereas K_{10} proved deficient. Interaction was also significant, the combination 100%×K_{20} proved optimum,

Table 50. Effect of GW, 50%WW and 100%WW on siliqua length (cm), siliqua number plant^{-1} and seeds siliqua^{-1} of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Siliqua length				Siliqua number			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	5.21	5.44	5.67	5.44	92.23	95.68	97.64	95.18
K_{10}	5.86	6.17	6.25	6.09	99.06	108.00	109.73	105.60
K_{20}	6.38	6.76	6.92	6.69	111.52	117.75	121.56	116.94
K_{30}	6.81	7.33	7.30	7.15	114.50	120.51	123.34	119.45
Mean	6.07	6.43	6.54		104.33	110.49	113.07	

	Seeds siliqua^{-1}			
	GW	50%WW	100%WW	Mean
K_0	13.54	13.97	14.24	13.92
K_{10}	14.39	14.76	15.00	14.72
K_{20}	15.11	15.58	15.76	15.48
K_{30}	15.35	15.66	15.82	15.61
Mean	14.60	14.99	15.21	

	CD at 5%		
	Siliqua length	Siliqua number	Seeds siliqua^{-1}
Water	NS	1.917	0.158
Potassium	0.454	2.914	0.182
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

Table 51. Effect of GW, 50%WW and 100%WW on 1000-seed weight (g), seed yield (g $plant^{-1}$) and biomass (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	1000-seed weight				Seed yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	5.03	5.17	5.26	5.15	6.98	7.13	7.52	7.21
K_{10}	5.40	5.57	5.68	5.55	7.83	8.21	8.56	8.20
K_{20}	5.54	5.80	5.86	5.73	8.50	9.82	10.25	9.52
K_{30}	5.60	5.85	5.88	5.78	9.03	9.90	10.30	9.74
Mean	5.39	5.60	5.67		8.09	8.77	9.16	

	Biomass			
	GW	50%WW	100%WW	Mean
K_0	11.78	12.20	12.77	12.25
K_{10}	13.03	14.28	14.71	14.01
K_{20}	14.52	15.59	16.00	15.37
K_{30}	14.81	15.85	16.19	15.62
Mean	13.54	14.48	14.92	

CD at 5%	1000-seed weight	Seed yield	Biomass
Water	0.063	0.226	0.338
Potassium	0.077	0.263	0.388
Interaction	NS	0.456	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

recording an increase of 46.85% over GW×K_0, it was also at par with 100%×K_{30}. Among other interactions 100%×K_{10} was at par with GW×K_{20}. In addition 100%×K_0 gave values at par to GW×K_{10} and 50%×K_0, again showing the effectiveness of the nutrients present in wastewater.

4.3.3.6 Plant Biomass

Wastewater produced plants with more biomass (Table 51). An increase of 10.19% was marked with 100%WW, whereas 50%WW marked an increase of 6.94% over GW. Among fertilizer doses, K_{20} increased the biomass upto 25.47% over K_0, whereas an increase of 14.37% was shown by K_{10}.

4.3.3.7 Oil Content

Effect of wastewater for oil content of seeds was found significant, however fertilizer effect proved non significant (Table 52). Although GW and 50%WW were equal in their effect, however value of 100%WW was different statistically and an increase of 2.66% was recorded by it.

4.3.3.7 Oil Yield Plant^{-1}

Wastewater also increased the oil yield of the plants (Table 52). 100%WW and 50%WW recorded different values thus marking an increase of 16.57%, 9.17% over GW. Like most other parameters observed earlier, here also K_{20} gave optimum value and recorded an increase of 35.33% over K_0. Its value was at par to K_{30}, again showing the luxury consumption of potassium under this treatment and K_{10} proved as deficient dose. Interaction was significant, 100%×K_{20} gave optimum value as it was at par with 100%×K_{30}. Further the values of 50%×K_{10} and GW×K_{20} were at par, showing utility of wastewater in terms of nutrient availability.

4.3.3.8 Seed Protein Content

Table 52 showed that WW helped in enhancing the protein content of seeds to some extent. Both 100%WW and 50%WW performed differently. An increase of 4.70% was shown by the former whereas latter recorded marginal increase of 2.94% over GW. Among various doses of potassium, K_{20} proved optimum by marking an increase of 10.11% over K_0. Interaction was non significant.

Table 52. Effect of GW, 50%WW and 100%WW on oil content (%), oil yield (g plant^{-1}) and seed protein content (%) of *Brassica juncea* cv. Varuna with different levels of potassium.

Treatments	Oil content				Oil yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
K_0	41.58	41.48	41.66	41.57	2.90	2.96	3.13	3.00
K_{10}	41.87	42.01	43.18	42.35	3.28	3.45	3.70	3.48
K_{20}	41.84	42.38	43.39	42.54	3.56	4.16	4.45	4.06
K_{30}	41.90	42.19	43.41	42.50	3.78	4.18	4.47	4.14
Mean	41.80	42.02	42.91		3.38	3.69	3.94	

	Seed protein content			
	GW	50%WW	100%WW	Mean
K_0	20.80	21.43	21.88	21.37
K_{10}	21.65	22.30	22.61	22.19
K_{20}	22.81	23.58	24.20	23.53
K_{30}	23.21	23.76	23.95	23.64
Mean	22.12	22.77	23.16	

CD at 5%	Oil content	Oil yield	Seed protein content
Water	0.81	0.060	0.278
Potassium	NS	0.082	0.321
Interaction	NS	0.134	NS

N.B.: Subscript values denote the amount of potassium (K) in kg ha^{-1}. A uniform dose of N_{80} and P_{30} in kg ha^{-1} was also applied basally.

4.4 EXPERIMENT -IV

The aim of this experiment was to confirm the findings of previous three experiments. In earlier experiments optimum doses of N, P and K were identified. However in this experiment optimum doses were given as full and half of the optimum so as to check if any fertilizer saving can be made under wastewater. Growth and physiological parameters were studied at vegetative, flowering and fruiting stages while yield and its attributes were taken at the time of harvest. Brief significant data were mentioned below.

4.4.1 Growth Parameters

Growth parameters like plant height, leaf number, leaf area, plant fresh weight and plant dry weight were studied.

4.4.1.1 Plant Height

Wastewater irrigation produced significantly taller plants over GW (Table 53). 100%WW recorded an increase of 11.39%, 14.34%, 12.57% over GW at three stages respectively followed by 50%WW where the percentage increase was 7.33, 9.30 and 8.16. The full combination of NPK fertilizers $N_{80}P_{30}K_{20}$ proved best by marking an increase of 36.22%, 47.34% and 39.45% over control. Interaction was significant only at flowering stage where 100%×$N_{80}P_{30}K_{20}$ gave maximum values by recording an increase of 65.37% over $N_0P_0K_0$, its value was at par to 50%×$N_{80}P_{30}K_{20}$. Among other interactions GW×$N_{40}P_{15}K_{10}$ and 100%×$N_0P_0K_0$ gave statistically equal values. Plant height increased with increase in age of the plants.

4.4.1.2 Leaf Number Plant^{-1}

WW irrigated plants produced more leaves than the plants irrigated with GW (Table 54). Both waters performed differently. An increase of 13.07%, 14.88%, 15.91% and 8.71%, 10.70%, 9.28% was recorded with 100%WW and 50%WW at three successive stages. Full dose of fertilizer proved best and recorded an increase of 40.08%, 37.78% and 39.16%. Interaction was significant at vegetative and flowering whereas at fruiting it was non significant. 100%×$N_{80}P_{30}K_{20}$ gave maximum values and recorded an increase of 57.43% and 53.93% over GW×$N_0P_0K_0$. At vegetative stage most of the interactions were critically different, but at flowering stage 100%×$N_{40}P_{15}K_{10}$ was at par with GW×$N_{80}P_{30}K_{20}$ and 50%×$N_{40}P_{15}K_{10}$. At par values

Table 53. Effect of GW, 50%WW and 100%WW on plant height (cm) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	25.40	26.80	27.90	26.70	43.43	45.06	48.20	45.56
$N_{40}P_{15}K_{10}$	29.31	31.60	32.92	31.28	50.60	55.51	57.00	54.37
$N_{80}P_{30}K_{20}$	34.10	36.90	38.10	36.37	60.81	68.70	71.82	67.11
Mean	29.60	31.77	32.97		51.61	56.42	59.01	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	59.10	62.24	64.50	61.95
$N_{40}P_{15}K_{10}$	68.26	73.10	77.18	72.85
$N_{80}P_{30}K_{20}$	79.52	88.44	91.20	86.39
Mean	68.96	74.59	77.63	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	1.95	1.82	1.91
Fertilizer	1.95	1.82	1.91
Interaction	NS	3.15	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 54. Effect of GW, 50%WW and 100%WW on leaf number plant^{-1} of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	14.00	14.85	15.30	14.72	25.81	27.00	27.72	26.84
$N_{40}P_{15}K_{10}$	16.08	17.31	17.95	17.11	28.00	31.05	32.36	30.47
$N_{80}P_{30}K_{20}$	18.81	21.00	22.04	20.62	33.08	38.14	39.73	36.98
Mean	16.30	17.72	18.43		28.96	32.06	33.27	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	22.13	23.71	24.18	23.34
$N_{40}P_{15}K_{10}$	25.23	27.56	29.90	27.56
$N_{80}P_{30}K_{20}$	29.55	32.80	35.09	32.48
Mean	25.64	28.02	29.72	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.355	1.012	1.26
Fertilizer	0.355	1.012	1.26
Interaction	0.614	1.750	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

were also shown by $GW \times N_{40}P_{15}K_{10}$, $100\% \times N_0P_0K_0$ and $50\% \times N_0P_0K_0$. Contrary to plant height, leaf number increased from vegetative to flowering stage and then it decreased.

4.4.1.3 Leaf Area Plant^{-1}

More leaf area was shown by the plants irrigated with WW (Table 55). An increase of 12.26%, 14.37% and 17.94% was given by 100%WW where as 50%WW marked an increase of 9.33%, 10.14% and 13.49%. Among fertilizer doses, $N_{80}P_{30}K_{20}$ gave maximum values by marking an increase of 41.23%, 46.47% and 54.86% whereas only an increase of 16.14%, 19.15% and 21.63% was shown by half fertilizer dose. Interaction was significant at flowering and fruiting stages where $100\% \times N_{80}P_{30}K_{20}$ gave maximum values by registering an increase of 63.50% and 77.74% over $GW \times N_0P_0K_0$. At both stages $GW \times N_{80}P_{30}K_{20}$ was at par with $100\% \times N_{40}P_{15}K_{10}$ but at fruiting it was also at par with $50\% \times N_{40}P_{15}K_{10}$. Like leaf number, leaf area also increased from vegetative to flowering stage only and after that it decreased.

4.4.1.4 Fresh Weight Plant^{-1}

Wastewater irrigation also increased the fresh weight of plants and critically different values were given by 100%WW and 50%WW at all the three stages studied (Table 56). An increase of 12.68%, 14.89% and 12.41% was recorded with the former over GW. Full fertilizer dose $N_{80}P_{30}K_{20}$ proved best by recording an increase of 41.68%, 41.64% and 33.02% whereas half dose $N_{40}P_{15}K_{10}$ was comparatively less effective, although it also recorded an increase of 18.69%, 18.48% and 14.05% over control. Interaction was significant at all the three stages and $100\% \times N_{80}P_{30}K_{20}$ gave the maximum values by recording an increase of 56.72%, 58.10% and 47.32% over $GW \times N_0P_0K_0$. However $GW \times N_{80}P_{30}K_{20}$ (full dose) was at par with $100\% \times N_{40}P_{15}K_{10}$ (half dose) at vegetative, flowering and fruiting stages highlighting the utilization of nutrients present in wastewater and proving effective use of wastewater by saving inorganic fertilizer. Plant fresh weight increased with increase in age of the plants. Increase was more from vegetative to flowering stage.

Table 55. Effect of GW, 50%WW and 100%WW on leaf area (cm^2 $plant^{-1}$) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	251.43	268.42	276.82	265.56	356.24	378.24	387.98	374.15
$N_{40}P_{15}K_{10}$	285.26	318.23	321.77	308.42	413.23	448.21	476.00	445.81
$N_{80}P_{30}K_{20}$	348.64	381.26	395.28	375.06	495.21	566.44	582.45	548.03
Mean	295.11	322.64	331.29		421.56	464.30	482.14	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	308.69	326.46	332.77	322.64
$N_{40}P_{15}K_{10}$	360.86	401.14	415.29	392.43
$N_{80}P_{30}K_{20}$	430.01	520.26	548.66	499.64
Mean	366.52	415.95	432.24	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	13.44	11.78	8.64
Fertilizer	13.44	11.78	8.64
Interaction	NS	20.90	14.96

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 56. Effect of GW, 50%WW and 100%WW on plant fresh weight (g plant^{-1}) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	8.48	8.84	9.18	8.83	14.75	15.41	16.09	15.42
$N_{40}P_{15}K_{10}$	9.85	10.46	11.13	10.48	16.81	18.62	19.39	18.27
$N_{80}P_{30}K_{20}$	11.48	12.76	13.29	12.51	19.62	22.58	23.32	21.84
Mean	9.94	10.69	11.20		17.06	18.87	19.60	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	23.50	24.88	25.47	24.62
$N_{40}P_{15}K_{10}$	26.31	28.31	29.62	28.08
$N_{80}P_{30}K_{20}$	30.00	33.64	34.62	32.75
Mean	26.60	28.94	29.90	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.372	0.268	0.465
Fertilizer	0.372	0.268	0.465
Interaction	0.645	0.421	0.812

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

4.4.1.5 Dry Weight Plant^{-1}

As evident from the table 57 that WW application lead to more dry weight accumulation over GW. 100%WW recorded an increase of 12.97%, 13.12%, 12.92% whereas an increase of 6.65%, 9.04% and 9.66% was marked with 50%WW. Interaction was significant at flowering and fruiting stages and 100%×$N_{80}P_{30}K_{20}$ proved best dose by registering an increase of 52.75% and 48.54% respectively. Most other interactions were critically different. Like plant fresh weight, dry weight also increased with increase in age of the plants, however percentage increase was more from vegetative to flowering stage.

4.4.2 Physiological Parameters

Like earlier experiments, in this experiment also total chlorophyll content, carotenoids, nitrate reductase activity, carbonic anhydrase activity, leaf nitrogen, phosphorus and potassium contents were studied at vegetative, flowering and fruiting stages.

4.4.2.1 Total Chlorophyll Content

Wastewater increased the chlorophyll content of plants only at flowering and fruiting stages and at vegetative stage it was non significant (Table 58). An increase of 10.45%, 11.96% and 5.97%, 7.61% was shown by 100%WW and 50%WW respectively. $N_{80}P_{30}K_{20}$ recorded an increase of 35.88%, 37.02% and 38.32% over $N_0P_0K_0$. Like leaf number and leaf area, chlorophyll content also increased till flowering and then decreased at fruiting.

4.4.2.2. Carotenoids

As evident from the table 59 that carotenoid content of leaves was enhanced with the application of wastewater. Both concentrations performed differently at vegetative and flowering stages whereas at fruiting their performance was similar. An increase of 9.54%, 9.28%, 9.66% and 5.21%, 5.86%, 6.44% was shown by 100%WW and 50%WW respectively. Fertilizer dose $N_{80}P_{30}K_{20}$ gave maximum value by marking an increase of 26.87%, 33.09% and 29.71% over GW×$N_0P_0K_0$ Carotenoids followed the trend of chlorophyll contents and increased upto flowering stage.

Table 57. Effect of GW, 50%WW and 100%WW on plant dry weight (g $plant^{-1}$) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	2.68	2.80	2.92	2.80	4.91	5.15	5.30	5.12
$N_{40}P_{15}K_{10}$	3.13	3.41	3.54	3.36	5.55	6.06	6.33	5.98
$N_{80}P_{30}K_{20}$	3.66	3.90	4.24	3.93	6.46	7.25	7.50	7.07
Mean	3.16	3.37	3.57		5.64	6.15	6.38	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	7.85	8.40	8.52	8.26
$N_{40}P_{15}K_{10}$	8.76	9.66	9.97	9.46
$N_{80}P_{30}K_{20}$	10.09	11.23	11.66	10.99
Mean	8.90	9.76	10.05	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.133	0.145	0.146
Fertilizer	0.133	0.145	0.146
Interaction	NS	0.251	0.253

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 58. Effect of GW, 50%WW and 100%WW on total chlorophyll content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	1.26	1.31	1.35	1.31	1.75	1.81	1.87	1.81
$N_{40}P_{15}K_{10}$	1.41	1.50	1.59	1.50	1.96	2.08	2.16	2.07
$N_{80}P_{30}K_{20}$	1.68	1.80	1.86	1.78	2.31	2.50	2.64	2.48
Mean	1.45	1.54	1.60		2.01	2.13	2.22	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	1.60	1.68	1.73	1.67
$N_{40}P_{15}K_{10}$	1.80	1.92	2.00	1.91
$N_{80}P_{30}K_{20}$	2.13	2.35	2.46	2.31
Mean	1.84	1.98	2.06	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	NS	0.133	0.059
Fertilizer	0.125	0.133	0.059
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 59. Effect of GW, 50%WW and 100%WW on carotenoid content (mg g^{-1} fresh weight) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	0.410	0.430	0.445	0.428	0.531	0.550	0.578	0.553
$N_{40}P_{15}K_{10}$	0.456	0.482	0.502	0.480	0.610	0.658	0.671	0.646
$N_{80}P_{30}K_{20}$	0.518	0.544	0.567	0.543	0.701	0.742	0.764	0.736
Mean	0.461	0.485	0.505		0.614	0.650	0.671	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	0.492	0.518	0.535	0.515
$N_{40}P_{15}K_{10}$	0.556	0.589	0.608	0.584
$N_{80}P_{30}K_{20}$	0.630	0.679	0.695	0.668
Mean	0.559	0.595	0.613	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.019	0.020	0.019
Fertilizer	0.019	0.020	0.019
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

4.4.2.3 Nitrate Reductase Activity

Wastewater enhanced the NRA also and different effect was shown by the two concentrations at flowering stage (Table 60). An increase of 11.22%, 11.88% and 13.96% was shown by 100%WW over GW at the three successive stages of growth, however 50% marked an increase of 6.56%, 7.80% and 9.79%. Fertilizer dose $N_{80}P_{30}K_{20}$ recorded maximum values by registering an increase of 41.71%, 39.41% and 44.64%, whereas half fertilizer dose marked an increase of 18.75%, 17.87% and 18.60% over control. Interaction was non significant. NRA increased from vegetative to flowering stage and then decreased.

4.4.2.4 Carbonic Anhydrase (CA) Activity

100%WW proved beneficial in enhancing enzymatic activity at vegetative and fruiting stages, however at flowering no significant increase was observed (Table 61). An increase of 13.52%, 9.51% and 7.82%, 5.71% was recorded with 100%WW and 50%WW at vegetative and fruiting stages respectively but the performance of both these concentrations was similar. $N_{80}P_{30}K_{20}$ proved best by recording an increase of 40.00%, 33.86% and 29.97% over GW×$N_0P_0K_0$. Interaction was non significant. Like leaf number, leaf area and NRA, CA also increased upto flowering.

4.4.2.5 Leaf Nitrogen Content

Nitrogen content of the leaves irrigated with WW was considerably high and significantly different from the plants irrigated with GW (Table 62). Both concentrations of wastewater were at par at fruiting stage whereas at vegetative and flowering stages their performance was different. An increase of 11.92%, 10.98% and 12.26% was marked with 100%WW over GW. Better effect was shown by $N_{80}P_{30}K_{20}$ by marking an increase of 35.02%, 33.89%, 40.28% over $N_0P_0K_0$ at three successive stages. Interaction was significant at vegetative stage only where GW×$N_{80}P_{30}K_{20}$ gave value at par to 100%×$N_{40}P_{15}K_{10}$. Further 100%×$N_0P_0K_0$ gave value at par to GW×$N_{40}P_{15}K_{10}$ and 50%×$N_0P_0K_0$. Nitrogen content decreased with increase in age of the plants.

4.4.2.6 Leaf Phosphorus Content

WW application significantly increased it, and an increase of 11.90%, 11.89% and 12.50% was shown by 100%, where as 50%WW registered an increase of 8.13%,

Table 60. Effect of GW, 50%WW and 100%WW on nitrate reductase activity (μ mol g^{-1} leaf fresh weight h^{-1}) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	261.18	270.42	280.25	270.62	351.84	375.24	388.21	371.76
$N_{40}P_{15}K_{10}$	306.42	316.28	341.40	321.37	406.41	448.16	460.07	438.21
$N_{80}P_{30}K_{20}$	353.31	394.59	402.61	383.50	488.21	520.31	546.26	518.26
Mean	306.97	327.10	341.42		415.49	447.90	464.85	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	301.24	316.32	324.13	313.90
$N_{40}P_{15}K_{10}$	346.46	374.81	395.60	372.29
$N_{80}P_{30}K_{20}$	408.91	468.90	484.28	454.03
Mean	352.20	386.68	401.34	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	14.33	15.95	14.83
Fertilizer	14.33	15.95	14.83
Interaction	NS	NS	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 61. Effect of GW, 50%WW and 100%WW on carbonic anhydrase activity [mol (CO_2) kg^{-1} (leaf fresh mass) s^{-1}] of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	2.45	2.54	2.66	2.55	3.68	3.81	3.94	3.81
$N_{40}P_{15}K_{10}$	2.75	2.90	3.11	2.92	4.12	4.60	4.67	4.46
$N_{80}P_{30}K_{20}$	3.24	3.66	3.81	3.57	4.95	5.14	5.21	5.10
Mean	2.81	3.03	3.19		4.25	4.52	4.61	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
$N_0P_0K_0$	3.23	3.38	3.49	3.37				
$N_{40}P_{15}K_{10}$	3.62	3.88	4.05	3.85	Water	0.183	NS	0.194
$N_{80}P_{30}K_{20}$	4.18	4.41	4.55	4.38	Fertilizer	0.183	0.356	0.194
Mean	3.68	3.89	4.03		Interaction	NS	NS	0.335

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 62. Effect of GW, 50%WW and 100%WW on leaf nitrogen content (%) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	3.18	3.35	3.48	3.34	2.91	3.03	3.08	3.01
$N_{40}P_{15}K_{10}$	3.64	3.88	4.09	3.87	3.20	3.44	3.58	3.41
$N_{80}P_{30}K_{20}$	4.26	4.46	4.81	4.51	3.73	4.10	4.26	4.03
Mean	3.69	3.90	4.13		3.28	3.52	3.64	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	2.70	2.87	2.93	2.83
$N_{40}P_{15}K_{10}$	3.11	3.30	3.59	3.33
$N_{80}P_{30}K_{20}$	3.74	4.00	4.18	3.97
Mean	3.18	3.39	3.57	

	CD at 5%		
	Vegetative stage	Flowering stage	Fruiting stage
Water	0.167	0.189	0.183
Fertilizer	0.167	0.189	0.183
Interaction	0.290	NS	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

8.28% and 7.46% over GW (Table 63). While $N_{80}P_{30}K_{20}$ recorded an increase of 34.92%, 35.66% and 38.54% over control. Interaction was significant and 100%×$N_{80}P_{30}K_{20}$ gave maximum values by marking an increase of 47.53%, 49.27% and 52.67%. At vegetative stage most of the interactions were critically different, but at flowering and fruiting stages at par values were shown by GW×$N_{80}P_{30}K_{20}$ and 100%×$N_{40}P_{15}K_{10}$. Phosphorus content also decreased with increase in age of the plants.

4.4.2.7 Leaf Potassium Content

A significant increase was observed in this parameter also where the leaves irrigated with 100%WW marked an increase of 9.43%, 11.24% and 10.65% over GW. Fertilizer dose $N_{80}P_{30}K_{20}$ proved best and registered an increase of 32.25%, 35.11% and 36.07% over control (Table 64). Interaction was significant. At all the stages 100%×$N_{80}P_{30}K_{20}$ gave maximum values by recording an increase of 43.56%, 46.43% and 49.49% over controls. Like leaf nitrogen and phosphorus content, leaf potassium content also decreased with increase in age of the plants. As observed earlier, K contents were maximum followed by N and P.

4.4.3 Yield and Quality Parameters

Siliqua length, siliqua number plant^{-1}, seeds siliqua^{-1}, 1000 seed weight, seed yield plant^{-1}, plant biomass, oil content, oil yield, seed protein content and heavy metal contents were observed and data were discussed below.

4.4.3.1 Siliqua length

Wastewater produced comparatively longer siliquae over GW (Table 65). An increase of 7.25% was shown by 100%WW, whereas 50%WW recorded an increase of 4.38%. 50%WW was at par with GW on one hand, whereas on the other it was at par with 100%WW. Like most other parameters, $N_{80}P_{30}K_{20}$ proved best and recorded an increase of 21.00% which was 9.24% more of half fertilizer. Interaction was non significant.

4.4.3.2 Siliqua Number Plant^{-1}

Wastewater produced significantly higher number of siliquae over GW (Table 65). An increase of 10.65% and 7.01% was shown by 100%WW and 50%WW respectively. Fertilizer dose $N_{80}P_{30}K_{20}$ proved best by recording an increase of

Table 63. Effect of GW, 50%WW and 100%WW on leaf phosphorus content (%) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	0.446	0.460	0.478	0.461	0.412	0.432	0.444	0.429
$N_{40}P_{15}K_{10}$	0.492	0.540	0.556	0.529	0.465	0.503	0.521	0.496
$N_{80}P_{30}K_{20}$	0.573	0.636	0.658	0.622	0.535	0.595	0.615	0.582
Mean	0.504	0.545	0.564		0.471	0.510	0.527	

	Fruiting stage				CD at 5%			
	GW	50%WW	100%WW	Mean		Vegetative stage	Flowering stage	Fruiting stage
$N_0P_0K_0$	0.393	0.412	0.425	0.410				
$N_{40}P_{15}K_{10}$	0.448	0.481	0.514	0.481	Water	0.007	0.009	0.008
$N_{80}P_{30}K_{20}$	0.526	0.577	0.600	0.568	Fertilizer	0.007	0.009	0.008
Mean	0.456	0.490	0.513		Interaction	0.013	0.015	0.014

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 64. Effect of GW, 50%WW and 100%WW on leaf potassium content (%) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Vegetative stage				Flowering stage			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	3.26	3.38	3.50	3.38	3.08	3.22	3.27	3.19
$N_{40}P_{15}K_{10}$	3.65	3.84	4.00	3.83	3.34	3.73	3.81	3.63
$N_{80}P_{30}K_{20}$	4.21	4.53	4.68	4.47	3.98	4.45	4.51	4.31
Mean	3.71	3.92	4.06		3.47	3.80	3.86	

	Fruiting stage			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	2.93	3.06	3.15	3.05
$N_{40}P_{15}K_{10}$	3.34	3.56	3.70	3.53
$N_{80}P_{30}K_{20}$	3.87	4.20	4.38	4.15
Mean	3.38	3.61	3.74	

CD at 5%	Vegetative stage	Flowering stage	Fruiting stage
Water	0.056	0.099	NS
Fertilizer	0.056	0.099	0.13
Interaction	0.097	0.172	NS

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 65. Effect of GW, 50%WW and 100%WW on siliqua length (cm), siliqua number plant^{-1} and seeds siliqua^{-1} of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Siliqua length				Siliqua number			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	5.36	5.60	5.74	5.57	92.54	94.81	96.59	94.65
$N_{40}P_{15}K_{10}$	5.92	6.20	6.38	6.17	100.13	108.04	112.70	106.96
$N_{80}P_{30}K_{20}$	6.51	6.76	6.96	6.74	113.35	124.64	129.33	122.44
Mean	5.93	6.19	6.36		102.01	109.16	112.87	

	Seeds siliqua^{-1}				CD at 5%			
	GW	50%WW	100%WW	Mean		Siliqua length	Siliqua number	Seeds siliqua^{-1}
$N_0P_0K_0$	13.34	13.78	14.05	13.72				
$N_{40}P_{15}K_{10}$	14.11	14.61	15.09	14.60	Water	0.283	1.606	0.222
$N_{80}P_{30}K_{20}$	15.45	15.69	15.86	15.67	Fertilizer	0.283	1.606	0.222
Mean	14.30	14.69	15.00		Interaction	NS	2.782	0.384

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

29.36% over $N_0P_0K_0$, half dose marked only an increase of 13.00%. Like most of the parameters 100%×$N_{80}P_{30}K_{20}$ proved best by marking an increase of 39.76% over GW×$N_0P_0K_0$. Significantly, value of GW×$N_{80}P_{30}K_{20}$ was at par with 100%×$N_{40}P_{15}K_{10}$.

4.4.3.3 Seeds Siliqua^{-1}

WW increased the number of seeds siliqua^{-1} (Table 65). An increase of 4.90% and 2.73% was shown by 100% and 50%WW respectively. Fertilizer $N_{80}P_{30}K_{20}$ gave maximum value and recorded an increase of 14.21% over $N_0P_0K_0$. Interaction was significant with 100%×$N_{80}P_{30}K_{20}$ attaining maximum value of 18.89% over GW×$N_0P_0K_0$. However values were at par with 50%×$N_{80}P_{30}K_{20}$. Values of GW×$N_{80}P_{30}K_{20}$ were at par with 50%×$N_{80}P_{30}K_{20}$ on one hand and on the other with 100%×$N_{40}P_{15}K_{10}$. Further at par values were also shown by GW×$N_{40}P_{15}K_{10}$, 100%×$N_0P_0K_0$ and 50%×$N_0P_0K_0$ again showing the beneficial utilization of nutrients present in wastewater for this important seed yield contributing attribute.

4.4.3.4 1000 Seed Weight

No significant effect was observed in 1000 seed weight of the plants irrigated with WW (Table 66). However, significant role was played by fertilizer for this parameter. Full fertilizer dose recorded an increase of 14.04% whereas an increase of 5.19% was shown by half fertilizer dose over control. It may be also added that full fertilizer dose gave 8.40% more test weight over half dose of fertilizer.

4.4.3.5 Seed Yield Plant^{-1}

Wastewater application enhanced the seed yield of the plants (Table 66) 100%WW showed an increase of 14.18% whereas an increase of 9.66% was given by 50%WW. $N_{80}P_{30}K_{20}$ gave maximum value by recording an increase of 36.28% over $N_0P_0K_0$, which was 19.26% more than $N_{40}P_{15}K_{10}$ where the percentage increase was 14.27 over control. Although maximum percentage increase was given by 100%×$N_{80}P_{30}K_{20}$. The combination GW×$N_{80}P_{30}K_{20}$ gave value which was at par with 100%×$N_{40}P_{15}K_{10}$ proving that half fertilizer dose along with wastewater was as effective as full fertilizer dose with ground water achieving the objective of this trial where it was assumed that nutrients present in wastewater can effectively be utilized and a good amount of inorganic fertilizer and fresh water can be saved. Also value of

Table 66. Effect of GW, 50%WW and 100%WW on 1000-seed weight (g), seed yield (g plant^{-1}) and biomass (g plant^{-1}) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	1000-seed weight				Seed yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	5.11	5.21	5.28	5.20	7.01	7.45	7.63	7.36
$N_{40}P_{15}K_{10}$	5.33	5.48	5.60	5.47	7.81	8.50	8.93	8.41
$N_{80}P_{30}K_{20}$	5.77	5.95	6.08	5.93	9.09	10.26	10.74	10.03
Mean	5.40	5.55	5.65		7.97	8.74	9.10	

	Biomass			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	11.48	12.00	12.38	11.95
$N_{40}P_{15}K_{10}$	12.74	13.65	14.16	13.52
$N_{80}P_{30}K_{20}$	15.03	16.08	16.63	15.91
Mean	13.08	13.91	14.39	

CD at 5%	1000-seed weight	Seed yield	Biomass
Water	NS	0.196	0.148
Fertilizer	0.258	0.196	0.148
Interaction	NS	0.340	0.255

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

100%×$N_0P_0K_0$ was at par with GW×$N_{40}P_{15}K_{10}$, again highlighting the utility of wastewater and also showing the fertilizer economy.

4.4.3.6 Plant Biomass

Wastewater application also enhanced the biomass of plants (Table 66). An increase of 10.02% and 6.35% was shown by the plants irrigated with 100% and 50%WW respectively. For this parameter also $N_{80}P_{30}K_{20}$ gave maximum value and marked an increase of 33.14% over control. Among interactions 100%×$N_{80}P_{30}K_{20}$ gave higher value and marked an increase of 44.87% while the combinations of other interactions were critically different.

4.4.3.7 Oil Content

Oil content was significantly affected by wastewater in comparison to GW (Table 67) however fertilizer proved non significant. 50%WW was at par with 100%WW on one hand and on the other with GW. An increase of 4.01% was shown by 100%WW over GW.

4.4.3.8 Oil Yield

As evident from the table 67 that significant increase was found in the oil yield of plants irrigated with WW. 100%WW and 50%WW performed differently and marked an increase of 18.86% and 11.38% respectively over GW. Fertilizer dose, $N_{80}P_{30}K_{20}$ proved best by marking an increase of 39.80% over control. Interaction was also significant, thus 100%×$N_{80}P_{30}K_{20}$ proved best and gave significantly different and higher values from the other doses and registered an increase of 61.43% over GW×$N_0P_0K_0$. Among other interactions GW×$N_{80}P_{30}K_{20}$ gave value at par to 100%×$N_{40}P_{15}K_{10}$ thus confirming that just half fertilizer dose if given with wastewater will be equally effective with full fertilizer dose if given with ground water. This piece of information obtained while observing the interaction effect of fertilizer doses and water of both types. Further the values of 100%×$N_0P_0K_0$ were also at par to 50%×$N_0P_0K_0$ and GW×$N_{40}P_{15}K_{10}$.

4.4.3.9 Seed Protein Content

For the protein content of seeds wastewater did not show any significant increase (Table 67). But fertilizer doses $N_{80}P_{30}K_{20}$ and $N_{40}P_{15}$ K_{10} gave 9.72% and 4.03% more protein content over $N_0P_0K_0$. Significantly half fertilizer dos was at par with full fertilizer dose.

Table 67. Effect of GW, 50%WW and 100%WW on oil content (%), oil yield (g plant^{-1}) and seed protein content (%) of *Brassica juncea* cv. Varuna with different combinations of nitrogen, phosphorus and potassium.

Treatments	Oil content				Oil yield			
	GW	50%WW	100%WW	Mean	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	41.75	41.66	42.49	41.97	2.93	3.10	3.24	3.09
$N_{40}P_{15}K_{10}$	41.80	43.00	44.22	43.01	3.26	3.66	3.95	3.62
$N_{80}P_{30}K_{20}$	42.16	42.74	44.03	42.98	3.83	4.39	4.73	4.32
Mean	41.90	42.47	43.58		3.34	3.72	3.97	

	Seed protein content			
	GW	50%WW	100%WW	Mean
$N_0P_0K_0$	21.20	21.78	22.01	21.66
$N_{40}P_{15}K_{10}$	21.91	22.63	23.07	22.54
$N_{80}P_{30}K_{20}$	23.18	24.13	24.00	23.77
Mean	22.10	22.85	23.03	

CD at 5%	Oil content	Oil yield	Seed protein content
Water	1.56	0.088	NS
Fertilizer	NS	0.088	1.566
Interaction	NS	0.152	2.712

N.B.: Subscript values denote the amount of fertilizer in kg ha^{-1}.

Table 68. Effect of GW, 50%WW and 100%WW on cadmium (μg g^{-1}), chromium (μg g^{-1}), lead (μg g^{-1}) and nickel (μg g^{-1}) content in seeds of *Brassica juncea* cv. Varuna.

Treatments	Cadmium			Chromium			Lead			Nickel		
	GW	50%WW	100%WW	GW	50%WW	100%WW	GW	50%WW	100%WW	GW	50%WW	100%WW
N_0	ND	ND	ND	0.011	0.022	0.033	0.105	0.247	0.380	0.090	0.188	0.285
N_{40}	ND	ND	ND	0.013	0.022	0.032	0.108	0.246	0.370	0.085	0.192	0.288
N_{80}	ND	ND	ND	0.012	0.022	0.032	0.114	0.245	0.405	0.080	0.173	0.300
N_{120}	ND	ND	ND	0.012	0.021	0.033	0.112	0.245	0.390	0.010	0.173	0.295
P_0	ND	ND	ND	0.014	0.024	0.031	0.111	0.248	0.385	0.078	0.181	0.281
P_{20}	ND	ND	ND	0.012	0.024	0.032	0.112	0.250	0.371	0.075	0.186	0.281
P_{40}	ND	ND	ND	0.012	0.022	0.032	0.113	0.245	0.380	0.075	0.182	0.310
P_{60}	ND	ND	ND	0.013	0.022	0.032	0.112	0.247	0.365	0.086	0.186	0.303
K_0	ND	ND	ND	0.015	0.026	0.034	0.112	0.249	0.387	0.090	0.186	0.305
K_{10}	ND	ND	ND	0.014	0.026	0.035	0.113	0.252	0.390	0.081	0.185	0.300
K_{20}	ND	ND	ND	0.015	0.026	0.034	0.112	0.249	0.385	0.076	0.184	0.285
K_{30}	ND	ND	ND	0.015	0.026	0.034	0.113	0.245	0.395	0.076	0.178	0.288
$N_0P_0K_0$	ND	ND	ND	0.015	0.025	0.034	0.112	0.249	0.390	0.082	0.177	0.293
$P_{40}P_{35}K_{10}$	ND	ND	ND	0.019	0.025	0.034	0.112	0.251	0.390	0.084	0.175	0.300
$K_{80}P_{30}K_{20}$	ND	ND	ND	0.019	0.026	0.035	0.113	0.250	0.393	0.079	0.186	0.286

N.B.: Subscript values denotes the amount of fertilizer in kg ha^{-1}. ND = Not Detected.

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DISCUSSION

5.1 Plant nutrition through inorganic fertilizers

In a normal soil, all essential nutrients are generally present, however continuous cropping results in depletion of nutrients, especially the three macroelements N, P and K, from the soil which can not properly nourish crops without their supply (Russell, 1950; Donahue *et al.*, 1990). Thus, the judicious application of nutrients can play an indispensable role in the realisation of the full genetic potential of crop plants (Milthorpe and Moorby, 1979). The ameliorative effect of these nutrients is also evident from the present study (Experiments I-IV).

5.1.1 Nitrogen

In Experiment I, application of nitrogen in general and N_{80} in particular proved good for most of growth and yield parameters including seed and oil yield. Expectedly, it may be due to nitrogen which increased the number of meristmatic cells and growth leading to the formation of branches in addition to leaf expansion and number (Dwelle *et al.*, 1981; Kleinkopf *et al.*, 1981; Lawlor *et al.*, 1989). It is a necessary component of several key biomolecules (Salisbury and Ross, 1992), therefore, it has affected the proportion of organs and structure (Pearman *et al.*, 1977, Sinclair and Horie, 1989, Greenwood *et al.*, 1991). When its supply is suboptimal, the growth may remain retarded (Marschner, 2002) as was observed in the present study also where N_{40} proved deficient recording comparatively lower values for leaf number, leaf area, plant fresh weight and plant dry weight (Tables 9-12). Higher values in growth parameters were obtained when this nutrient was given at its optimum level. It is, therefore, logical to conclude that N_{80} enhanced the leaf production, leaf area, fresh and dry weight up to the maximum when applied with wastewater, as higher dose (N_{120}) could not enhance them further thus proving the "luxury dose".

It may further be noted that the supply of this nutrient also increased the total chlorophyll contents, carotenoids, NRA, and CA (Tables 13-16). Since chlorophylls being nitrogenous in nature consequently depend upon this element for their production. NRA helps in nitrate metabolism and its activity can be induced by the addition of nitrate nitrogen (Oaks *et al.*, 1972), whereas CA is responsible for the reversible hydration of CO_2 and is necessary for optimal photosynthetic activity (Everson, 1970) and its activity declines within few days if nitrogen is deficient (Marschner, 2002) as may be observed under lower dose (Table 16). The leaf analysis showed enhanced NPK contents under nitrogen application. The easy availability of these essential nutrients from the soil (Table 4), wastewater (Table 5) and inorganic fertilizers (Tables 1 and 2) may lead to the higher uptake of these nutrients by plant roots and their unhindered distribution to the tops. The highest contents of nitrogen and potassium were recorded at optimum level of nitrogen (Table 17 and 19) and of phosphorus at N_{120} (Table 18). Such pattern of nutrient contents may be ascribed to the synergistic effect of nutrients. This relationship of nitrogen and phosphorus has also been reported by Russell (1973) and between nitrogen and potassium by Murphy (1980).

Application of N_{80} resulted in maximum seed yield (Table 21). High grain yield can be achieved only when proper combination of variety and agronomic practices are obtained. The processes involved in seed production like vegetative growth, formation of storage organs and seed filling help in determining the seed yield (Yoshida, 1972). Therefore, increase in leaf area (Table 10) and leaf number (Table 9) may be cited as the two important growth parameters responsible for increase in seed yield under this treatment as the higher leaf area would be able to capture more radiant energy required for the enhanced photosynthetic activity as observed earlier (Tabassum, 2004). This was also evident by the positive correlation coefficient values between leaf area and seed yield at vegetative ($r = 0.996^{**}$), flowering ($r = 0.994^{**}$) and fruiting ($r = 0.991^{**}$) stages. Increase in NRA could serve another criterion relating to nitrogen metabolism and grain yield (Zeiserl *et al.*, 1963). Which was also strengthened by correlation between NRA and seed yield ($r = 0.990^{**}$, 0.991^{**} and 0.993^{**}) at three stages. In addition, nitrogen also enhanced the yield attributing characters individually which were cumulatively responsible for higher seed yield (Tables 20, 21). The correlations between some of the yield parameters and seed yield also confirmed this observation where r value was 0.999^{**} for siliqua number plant^{-1}, 0.990** for 1000-seed weight and 0.986** for seeds siliqua^{-1}. References were also available on mustard where nitrogen application enhanced the seed yield and its attributes however the doses applied were higher than N_{80} (Arthamwar *et al.*, 1996, Tomer *et al.*, 1997 and Bhari *et al.*, 2000). Contrary to their observations, in the present study, comparatively lower dose proved optimum due to the presence of two forms of nitrogen in the wastewater and thus saving some of the nitrogen. Unlike to growth and yield parameters, the oil content of seeds increased under N_{40} and decreased with higher nitrogen doses (Singh *et al.*, 2002; Pandey and Bharti, 2005). It may be because of preferential utilization of carbon skeletons at the time of seed filling towards protein synthesis than oil formation (Mazur *et al.*, 1977, Chourasia *et al.*, 1992). However, the positive effect of nitrogen on seed yield was so pronounced that it outbalanced the oil content value in providing the enhanced oil yield under N_{80} (Figs. 3, 5). The increase in seed protein content at N_{120} may be because of the direct role of nitrogen in protein formation, however protein content obtained under N_{80} was only 2.45% less than that of N_{120}.

5.1.2 Phosphorus

It is indispensable for all forms of life because of its role in energy transfer via ATP. Its compounds play major roles in the formation of various substances. In natural conditions, phosphorus is the limiting element due to its low availability and thus its application is desirable for the better harvest. In Experiment II, it was supplied in the form of mono-calcium super phosphate due to the easy availability of H_2PO_4 which may be taken up more readily than HPO_4 released from diammonium phosphate (Hagen and Hopkins, 1955). Among different doses, P_{30} proved optimum as it enhanced leaf number (Table 24) leaf area (Table 25) and plant dry weight (Table 27). The adequate supply of this element may be responsible for the enhanced vigour and leaf development leading to more leaf area (Patnaik, 1987), while the deficient dose may be responsible for the decrease in cell size and leaf area (Fredeen *et al.*, 1989), number of leaves (Lynch *et al.*, 1991) and dry matter accumulation (Nandal *et al.*, 1987) as observed under P_{15} in experiment II even in presence of wastewater.

Application of phosphorus also increased carotenoids (Table 29) as these are synthesised by isoprenoid unit which themselves are produced by the mediation of the phosphorus containing enzyme geranyl geranyl diphosphate (GGPP) synthase (Buchanan *et al.*, 2004). In contrast to the direct role of nitrogen in NR activity, phosphorus in leaf tissues is known to be responsible for phosphorylation and release of photosynthates from chloroplasts and oxidation of these sugars produces more reducing powers for nitrate metabolism (Kow *et al.*, 1982). Application of phosphorus also enhanced the uptake of N, P and K contents as positive correlation between P and N has been reported by Andrew and Robins (1969) and between P and K by Dev (1965). The increased leaf area (Table 25) resulted in increased photosynthates as observed by Tabassum (2004) and earlier Nair (1972), Osman *et al.* (1977) and Longstreth and Nobel (1980). The enhanced supply of photosynthates may result in the observed high seed yield. The optimum dose of phosphorus was also promoted the development of reproductive organs leading to the enhanced seeds siliqua^{-1} (Table 35), siliqua number plant^{-1} (Table 35), siliqua length (Table 35) and therefore, the seed yield (Fig. 3). This nutrient is also known to facilitate the partitioning of photosynthates between source and sink (Giaquinta and Quebedeaux, 1980) leading to higher 1000-seed weight (Table 36). Thus higher 'r' values are shown by siliqua number plant^{-1} ($r = 0.996^{**}$), seeds siliqua^{-1} ($r = 0.992^{**}$) and 1000-seed weight ($r = 0.987^{**}$) when correlations were made with seed yield. However, phosphorus was ineffective for promoting the oil content (Table 37) although there are reports where phosphorus application has enhanced the oil content (Arthamwar *et al.*, 1996; Patel and Shelke, 1998). It may be because of the dilution with growth effect in this study. However, it may be pointed out that inspite of the unaffected oil content, oil yield has been computed higher due to higher seed yield (Table 36).

5.1.3 Potassium

In Experiment III, it was applied to assess its requirement for mustard when given with wastewater. Therefore, application of the comparatively lower dose (K_{20}) resulted in the best results for various growth parameters (Tables 39, 40, 41). Obviously, the role of potassium on the phloem transport of sucrose may be responsible for K induced increase in the growth rate. The observed higher leaf area

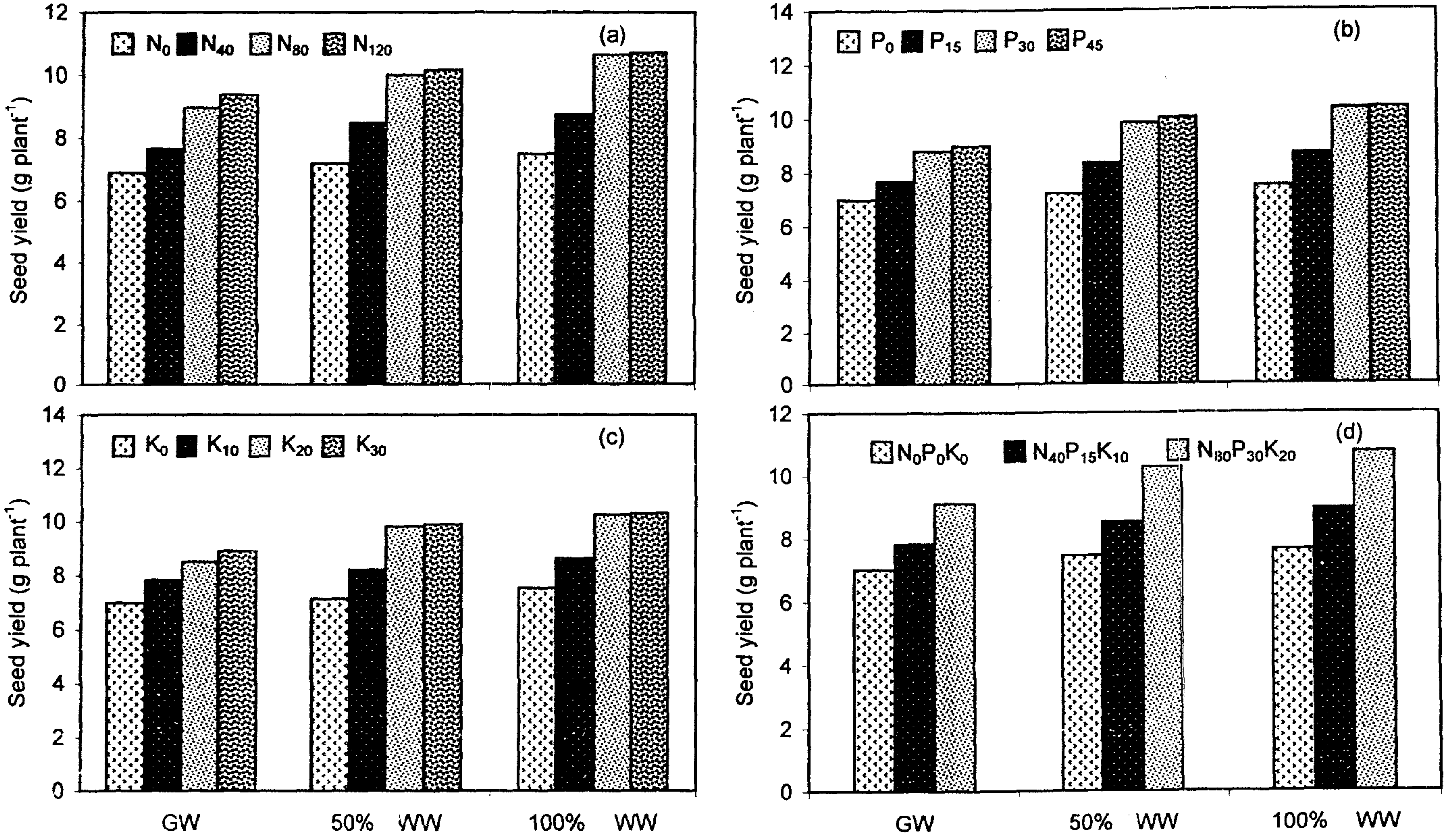

Fig. 3. Seed yield of *Brassica juncea* var. Varuna in Experiments I(a), II(b), III(c) and IV(d).

(Table 40) may be ascribed to its role in augmenting the cell size (Mengel and Arneke, 1982). Potassium besides being the activator or co-factor of various enzymes (Evans and Sorger, 1966) is dominant counterion to light induced H^+ influx across thyllakoid membranes (Tester and Blatt, 1989) and for establishment of pH gradient necessary for ATP synthesis. Moreover, it increases the rate of photosynthesis and RUBP carboxylase activity as well as photorespiration due to strong CO_2 depletion at catalytic sites of the enzyme. Thus, the enhanced leaf area and photosynthetic rate may ensure the higher dry matter production (Table 42).

Potassium being involved in peptide bond synthesis (Webster, 1959) and in other energy releasing processes (Evans and Sorger, 1966), would be expected to enhance NRA in leaf tissues (Table 45). It also enhanced NPK contents which may be explained (Noggle and Fritz, 1986) by increasing the external concentration of K. It may be pointed out that variations in mineral contents including N and P are influenced by various factors, including the type of crop and varieties, growth habits of root systems, the ability to absorb nutrients present near root zone and the chemical composition of the medium in which the plant is grown.

It may be recalled that K_{20} proved optimum for most of the growth parameters including leaf area. Thus, it is logical to conclude that this augmented source (leaf area) would have led to the better development of sink which was aptly demonstrated by enhanced siliqua plant^{-1} (Table 50), seeds siliqua^{-1} (Table 50) and 1000-seed weight (Table 51). The higher values for these parameters has culminated into the higher seed yield (Table 51). Correlation studies also showed the strong positive correlations of siliqua plant^{-1} ($r = 0.979^{**}$), seeds siliqua^{-1} ($r = 0.973^{**}$) and 1000-seed weight ($r = 0.965^{**}$) with seed yield. It may also be of interest to note that K_{30} seemed to be at luxury consumption as further enhancement in growth and development was not observed under this dose. Like phosphorus, potassium also proved ineffective for increasing the oil content, but there was an increase in oil yield due to the enhanced seed yield under the optimum dose of potassium (Figs. 3, 4, 5). Seed protein content was also improved due to the optimum K dose (Table 52) which can be attributed to its role in activation of enzymes involved in protein synthesis.

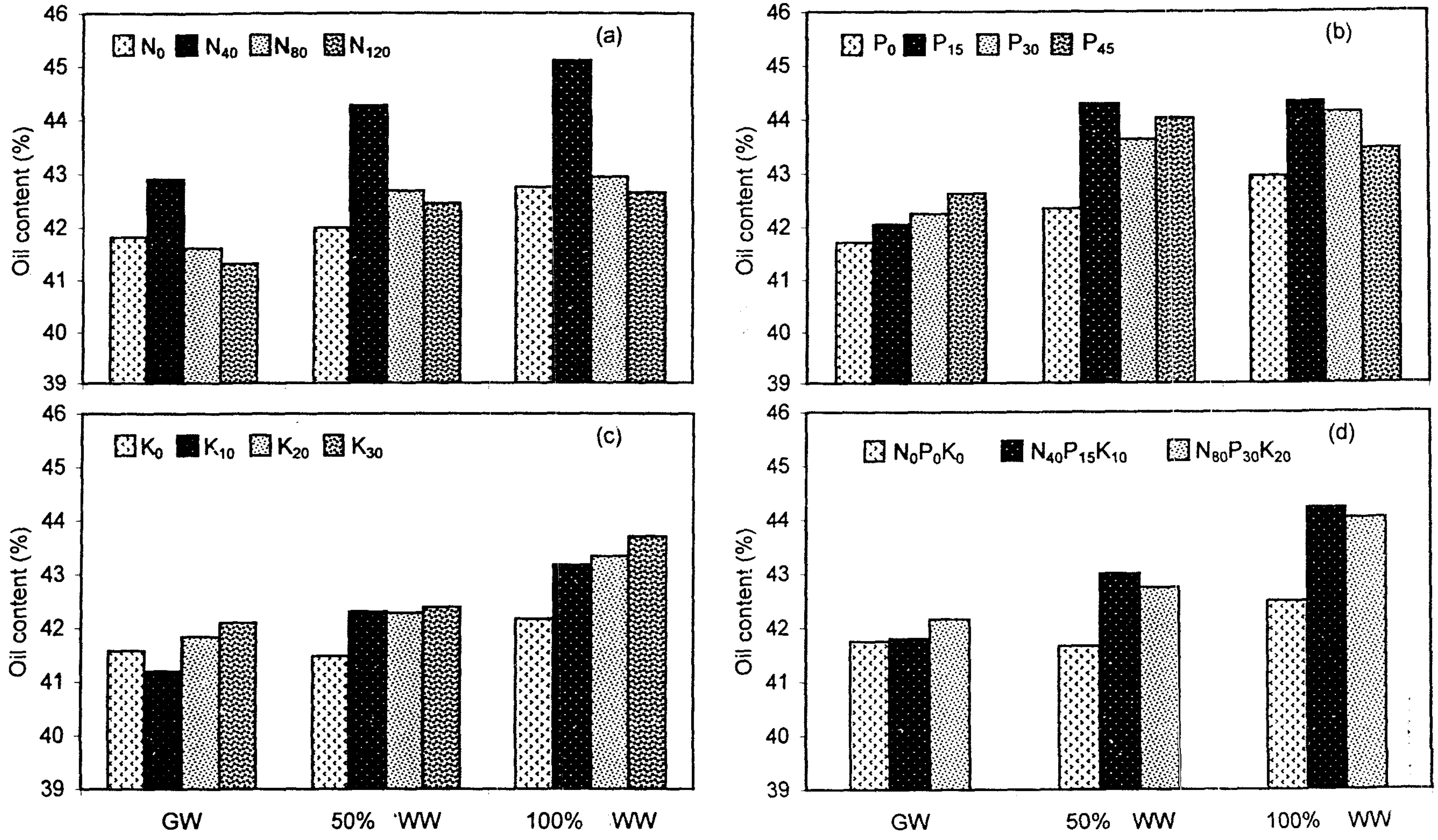

Fig. 4. Oil content of *Brassica juncea* var. Varuna in Experiments I(a), II(b), III(c) and IV(d).

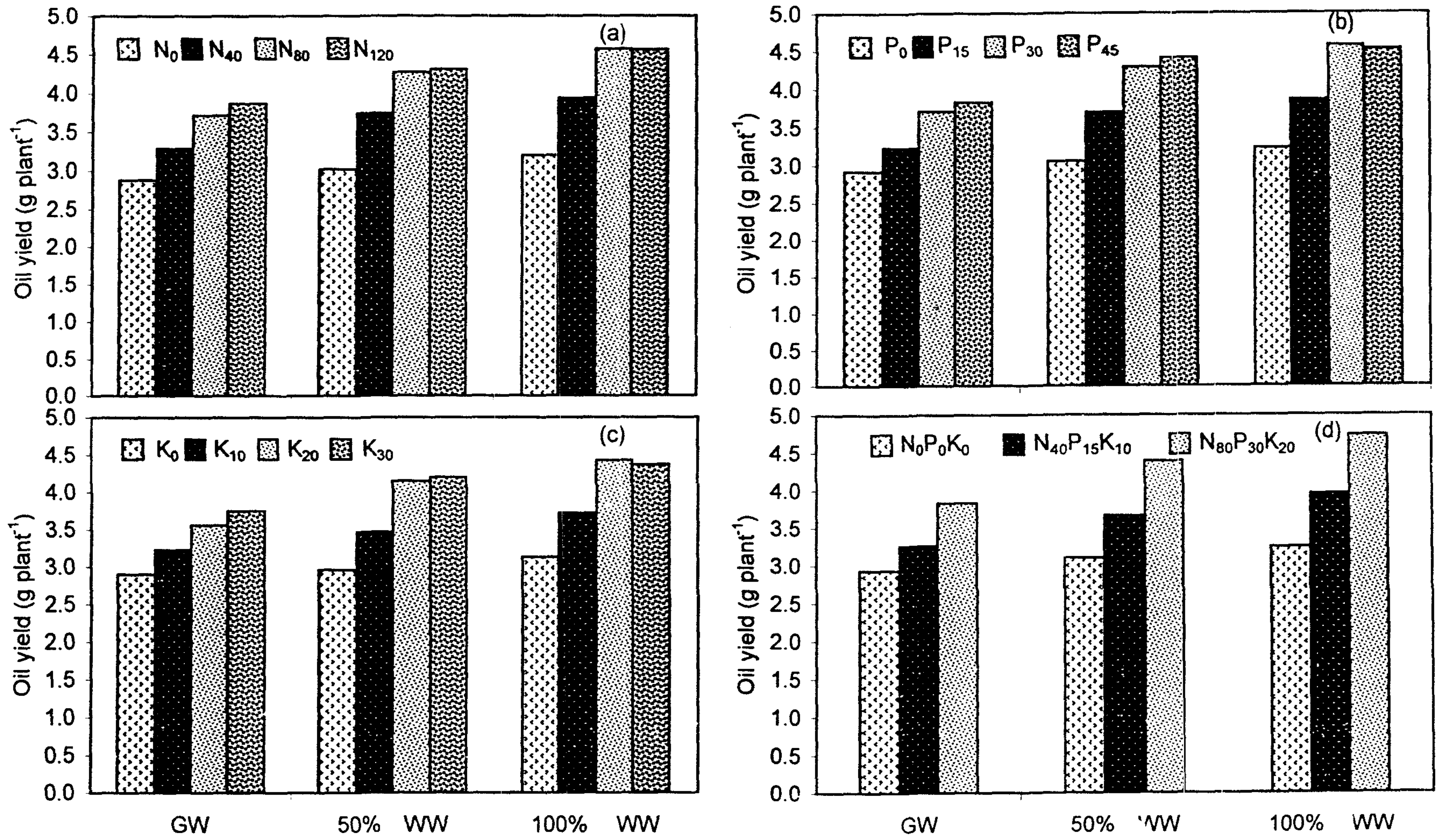

Fig. 5. Oil yield of *Brassica juncea* var. Varuna in Experiments I(a), II(b), III(c) and IV(d).

In Experiment IV, the combined optimum dose of NPK (determined in Experiments I-III) and its half dose were applied under wastewater and $N_{80}P_{30}K_{20}$ proved superior to the half dose for most of the growth and yield parameters. Data revealed that there was a cumulative effect of N, P and K when applied together at their optimum. In this regard the reference may be made of Russell (1973) who pointed out "if two factors are limiting or nearly limiting growth, adding only one of them will have little effect on growth, whilst adding both together will have considerable effect. Two such factors are said to have a large positive interaction in such circumstances for the response of the crop to both together is larger than the sum of responses of each separately". When interaction (wastewater×fertilizer) effect was taken into consideration, it was found that the effect of GW×$N_{80}P_{30}K_{20}$ was equalled by that of half dose wastewater×$N_{40}P_{15}K_{10}$ for most of the growth and yield parameters. This observation has proved the objectives of this study as the doses of inorganic fertilizers can be reduced substantially by the application of wastewater with an alternative way of wastewater disposal and its use in crop cultivation. These observations were in accordance with the findings of Nagaraja and Krishnamurthy (1989), Reboll *et al.* (2000) and Pradhan *et al.* (2001) who were of the opinion that lower doses of fertilizers may be applied with the wastewater for better plant growth.

5.2 Plant nutrition through the wastewater irrigation

As evident from Chapter 4 that wastewater was beneficial for better performance of the crop and 100%WW was more effective than 50%WW (Experiments I-III). The effectivity of 100% WW over 50% was because of the reason that it was diluted at several places before reaching to the main drain. While the suitability of waste water in general was because of some essential nutrients like N, P, K, S, Ca, Mg, Cl and even Ni (Table 5) which were present and available due to the wastewater application. Roles of NPK in growth and development have already been discussed and other nutrients have also some well established roles. It may be pointed out that sulphur deficiency is common in the world (Murphy and Boggan, 1988). Application of nitrogen in the form of urea is ineffective unless sulphur is applied simultaneously as its deficiency reduces the leaf area (Wang *et al.*, 1976, Burke *et al.*,

1986) besides decreasing the chlorophyll contents (Marschner, 2002). Among oilseed crops, rapeseed mustard has the highest requirement for this nutrient (Tandon, 1986) as it is an important constituent of its oil (Mengel and Kirkby, 1996). Therefore, the wastewater proved beneficial for oil content due to the presence of sulphate ions as sulphur favourably increases the conversion of sugars into oil. Similar increase in oil content of mustard with sulphur application has also been reported by Tomer *et al.* (1997), and Ahmad *et al.* (1998). In addition to this it is an important constituent of seed protein aminoacids (Tisdale and Nelson, 1975; Holmes, 1980) that is why 100%WW being rich in sulphur improved the seed proteins also, which may be useful for animal feed. Among other nutrients, Ca^+ being an essential component of cell wall is involved in cell division (Schmit, 1981) and Mg^{2+} a central atom of chlorophyll molecule is required for the structural integrity of chloroplasts (Moorby and Besford, 1983) on which rate of photosynthesis directly depends. While chlorine plays an essential role in stomatal regulation and its deficiency may be responsible in the reduction of leaf surface area and dry weight (Marschner, 2002). It may be noted that Ni has recently been added to the list of essential nutrients and its deficiency leads to depressed seedling vigour, chlorosis and necrotic lesions in leaves (Dalton *et al.*, 1988). Since the crop was watered on alternate days with the nutrient rich wastewater from which such nutrients may be absorbed by roots leading to better growth and development of the crop (Experiments I-IV).

It is worth mentioning that $GW \times N_{80}$ also (N_{120}) and 100% $WW \times N_{40}$ in Experiment I, $GW \times P_{45}$ and 100% $WW \times P_{15}$ in Experiment II, $GW \times K_{20}$ and 100%$\times K_{10}$ for seed yield, $GW \times K_{20}$ and 50% $WW \times K_{10}$ for oil yield and $GW \times N_{80}P_{30}K_{20}$ and $WW \times N_{40}P_{15}K_{10}$ (Experiment IV) were equally effective for seed and oil yield. This equal effectiveness of GW×nutrient/s and 100% wastewater × nutrient/s suggests that the wastewater is suitable for making nutrients available to the crop.

Heavy metal accumulation may occur in plants grown under wastewater. In recent years, it was observed that few plant species have the ability to accumulate comparatively higher amount of heavy metals without disturbing much growth and development (Reeves and Brooks, 1983; Brooks and Malaisse, 1985; Baker and Brooks, 1989). *Brassica juncea* is one of the hyper accumulator crop species therefore, seeds were also tested for some heavy metals like Cd, Cr, Pb, and Ni. Fortunately, their concentration was within the permissible limits (Pendias and Pendias, 1984), thus rendering the wastewater application nutritionally sustainable. In addition, the wastewater was also tested for microbes as the sewage water which was the part of this city wastewater have some pathogenic and non-pathogenic bacteria which, if cross the limits, can harm the farmer as well as consumer (Table 7). Since the crop is neither a vegetable nor eaten raw so chances of diseases are comparatively negligible to the consumer. However, it must be admitted that the growers may be warned to be careful during the irrigation operations of their crops.

5.3 Plant growth response

While taking the plant height (Tables 8, 23, 38, 53), plant fresh weight (Tables 11, 26, 41, 56) and plant dry weight (Tables 12, 27, 42, 57) into consideration, it was found that these parameters increased with the increase in age. This phenomenon is common in growth of plants. The increase was comparatively more from vegetative to flowering which may be because of sigmoid pattern where growth is comparatively faster in the log phase. While leaf number and leaf area were increased upto flowering only. This increase may be due to the senescence of older leaves as the concentration of nutrients in young leaves is maintained through the transport from older leaves leading to their senescence (Greenway and Gunn, 1966). In addition to this, absorption capacity of underground parts decreases as the plant ages thus creating discernible constraint at the later stages of growth where competition for nutrients and other inputs becomes more intense.

Total chlorophyll contents (Tables 13, 28, 43, 58), carotenoids (Tables 14, 29, 44, 59), NRA (Tables 15, 30, 45, 60) and CA (Tables 16, 31, 46, 61) showed increasing trend upto flowering only which may be due to decreasing density of photosynthetic pigments and enzymes per unit leaf area as the plant grows more (Bhagsari and Brown, 1986). Contrary to above observations, the continuous decrease was observed in leaf NPK contents with the increase in age. This decreasing trend can be ascribed to the exponential increase in growth (weight and volume) and as a result of "dilution with growth effect", even higher quantities of nutrients appear to be less when expressed on per unit basis (Moorby and Besford, 1983). Besides this, the translocation of nutrients towards seeds during their formation may also deplete leaf nutrient contents at the later stages. Among the three nutrients, K content was comparatively more in leaves (Tables 19, 34, 49, 64) followed by N (Tables 17, 32, 47, 62) and P (Tables 18, 33, 48, 63). This distribution of potassium may be due to its higher mobility, permeability and uptake (Mengel and Kirkby, 1996) and higher abundancy in plant tissues (Huber, 1985).

5.4 Conclusions

The following concluding points were emerged:

1. Nitrogen dose N_{80} proved optimum, N_{120} generally at luxury consumption and N_{40} as deficient except for oil content when its doses alone were considered. N_{80} interacted with 100% wastewater (100%×N_{80}) effectively among the interactions.
2. Phosphorus at P_{30} proved optimum and P_{45} generally at luxury consumption whereas P_{15} was deficient and for interactions P_{30} was effective with wastewater (100%×P_{30}).
3. Potassium at K_{20} was best and the higher and lower doses proved luxury and deficient respectively. In case of interactions K_{20} was effective with the wastewater (100%×K_{20}).
4. Considering the two doses of NPK, full dose ($N_{80}P_{30}K_{20}$) proved more effective than the half dose ($N_{40}P_{15}K_{10}$) for most of the growth and yield parameters.
5. However, on analyzing the interaction effect, half dose of NPK with 100% wastewater (100%×$N_{40}P_{15}K_{10}$) gave values equal to those of full dose and ground water (GW×$N_{80}P_{30}K_{20}$) proving the utility of wastewater as a source of these nutrients although the most effective combination was 100%×$N_{80}P_{30}K_{20}$ therefore, on the basis of four experiments this dose may be recommended to the grower of this crop under the local agroclimatic conditions of Aligarh.
6. Application of the wastewater can certainly reduce the excessive use of inorganic fertilizers therefore, proving good for the prevention of freshwater degradation due to eutrophication.

7. 100% WW proved more effective than 50% WW because of the higher amount of nutrients present in it.
8. Although the work undertaken earlier (Review of Literature) showed that the diluted wastewater proved effective in many cases in comparison with the raw wastewater but in the present study, 100% WW proved best, therefore, it can be used directly without any dilution.
9. Physico-chemical analysis of the wastewater revealed its suitability for irrigation because the values were within the permissible limits of the FAO and CPCB. Generally irrigation waters have conductivities less than 2250 μ mhos cm^{-1}. In the present study the conductivity of the irrigation water was upto 1400 μ mhos cm^{-1}, therefore it may be classified as medium to high saline and thus safe for irrigation (Mahida, 1981). Similarly, heavy metals Cd, Cr, Pb and Ni were also analysed in the wastewater and except Ni values of Cr, Cd and Pb were within the permissible limits of irrigation water (Gupta *et al.*, 2000).
10. Some pathogenic microorganisms like faecal coliforms, salmonella, shigella sp. were present in wastewater. Since the crop is not eaten uncooked therefore, there may not be any harm to the consumer and there are no standards. However, growers may be warned to be careful during irrigation operation.
11. Soil also contained some essential nutrients like N, P, K, S, Mg, Ca, Cl having sandy loam texture and medium alkaline pH, suitable for the availability of nutrients. It was also tested for the heavy metals mentioned above which were also within limits (Smith, 1996).
12. Plant height, fresh weight and dry weight increased with the increase in the age while leaf number, leaf area, total chlorophyll contents, carotenoids, NRA and CA increased up to flowering only and leaf NPK contents decreased with the increase in age of the plants.
13. K accumulated more in plants followed by N and P.
14. Heavy metals were also tested in seeds surprisingly Cd was not detected in any of the samples and others did not cross the limits (Pendias and Pendias, 1984).

15. Fertilizer proved comparatively less significant in increasing the oil content but the wastewater proved more effective. However oil yield was increased due to the fertilizers as well as the wastewater.
16. The wastewater also increased the protein content of seeds.

5.5 Proposal for future studies

Observations recorded during three years have helped in understanding the utility of the wastewater in crop production however, still there remain some areas where work can further be undertaken.

1. Experiments should be repeated in the farmers field near the drain to confirm the findings of pot experiments.
2. Due to the limited facility only a few heavy metals were tested in soil, water and seeds so in future study other heavy metals may be estimated in leaf, straw and oil cake.
3. Oil quality parameters, like acid, iodine and saponification values may also be tested under wastewater application.
4. Fatty acid composition of oil may be included in any future study.

SUMMARY

Chapter 6

SUMMARY

Chapter I (Introduction) included the justification of the research work undertaken. Importance of the plant nutrition, wastewater management through agriculture and crop tested was explained.

Before the beginning of Chapter II, a list of plants and their botanical names was included for the convenience of the reader.

Chapter II contained the review of literature on the effect of the wastewater on plants, NPK requirement of mustard and heavy metal accumulation in plants due to wastewater application.

In Chapter III, the methods and techniques employed in four pot experiments during the 'rabi' seasons of 2002-2005 were explained. Agroclimatic conditions of Aligarh, water and soil analysis including heavy metals and the wastewater microbiology were also incorporated.

Chapter IV dealt with experimental results which were presented in Tables 8-67 and summarised below.

Experiment I was conducted during the 'rabi' season of 2002-2003 to study the comparative effect of two concentrations of the city wastewater, i.e. 50%WW and 100%WW and ground water (control) on *Brassica juncea* var. Varuna grown with four levels of nitrogen, i.e. N_0, N_{40}, N_{80} and N_{120}. 100%WW proved beneficial for most parameters studied including seed and oil yield. Among different doses, N_{80} proved optimum as N_{120} was at luxury consumption and N_{40} was deficient. Interaction 100%×N_{80} proved good for most parameters. Experiment II was performed simultaneously with Experiment I to study the effect of wastewater treatments (as above) in presence of four levels of phosphatic fertilizer i.e. P_0, P_{15}, P_{30} and P_{45} on performance of the same crop. In this experiment also 100%WW proved best for most parameters studied. Application of P_{30} proved optimum, P_{45} at luxury consumption and P_{15} was deficient. Interaction, 100%×P_{30} proved superior over others.

In experiment III (2003-2004), the performance of the same variety of *Brassica juncea* was studied under the same 50%WW, 100%WW and GW in presence of four levels of potassium, i.e. K_0, K_{10}, K_{20} and K_{30}. Again 100% wastewater proved best. K_{20} gave better results while K_{30} was at luxury consumption and K_{10} was deficient. Interaction 100%×K_{20} surpassed the others.

In view of the fertilizer economy, effect of wastewater treatments (as in Experiments I-III) in presence of the combined optimum doses of nutrients determined in Experiments I-III ($N_{80}P_{30}K_{20}$) and the half of the combined optimum doses ($N_{40}P_{15}K_{10}$) was studied on the performance of the same crop in Experiment IV. Application of 100% WW again proved best. The combined optimum doses of the nutrients surpassed the half dose of nutrients. Significantly, effect of 100% WW with $N_{40}P_{15}K_{10}$ was at par with that of GW×$N_{80}P_{30}K_{20}$. This observation indicated the possibility of inorganic fertilizer saving if the wastewater is used for irrigation. Since 100%WW proved best therefore, there is no need of dilution which is incidentally common practice at Aligarh.

In Chapter V, the data were discussed in the light of the research work carried out by other workers on similar aspects and correlation analysis of some parameters with seed yield was also undertaken. In the end, conclusions were drawn and finally some suggestions were also incorporated for the future work. The present chapter gives the chapter wise glimpse of the entire study. It was followed by relevant references cited in the text and an appendix of the reagents used during the experimental work.

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APPENDIX

APPENDIX

1-Amino-2 naphthol-4 sulphonic acid

0.5g 1-Amino-2 naphthol-4 sulphonic acid dissolved in 195ml 15% sodium bisulphate solution to which 5ml 20% sodium sulphite solution was added.

Alkali iodide azide reagent

50g sodium hydroxide and 15g potassium iodide diluted to 100ml with double distilled water (DDW). 1g sodium azide dissolved in 4ml of DDW and added to the above solution.

Ammonium acetate solution (1N)

57ml glacial acetic acid was diluted to 800ml DDW and neutralized to pH 7.0 with concentrated ammonium hydroxide and final volume was made upto 1000ml.

Ammonium chloride–Ammonium hydroxide buffer

(a) 16.9g ammonium chloride dissolved in 142ml concentrated ammonium hydroxide (b) 1.179g of disodium EDTA and 0.780g magnesium sulphate dissolved in 50ml DDW. Both (a) and (b) solution mixed and diluted to 250ml with DDW.

Ammonium molybdate solution (2.5%)

(a) 25.0g ammonium molybdate dissolved in 175ml DDW (b) Add 280ml concentrated H_2SO_4 to 400ml DDW and cool. Mix the two solutions (a) and (b) and final volume made upto 1 litre with DDW.

Ammonium purpurate

150mg ammonium purpurate dissolved in 100g ethylene glycol.

Bromothymol blue indicator (0.02%)

0.002g bromothymol blue dissolved in 100ml DDW.

Conditioning reagent

50 ml of glycerol mixed in a solution containing 30ml concentrated HCl + 300ml DDW + 100ml 95% ethyl alcohol and 75g sodium chloride.

Cystein hydrochloride solution

48g cystein hydrochloride dissolved in sufficient DDW and final volume made upto 1000ml with DDW.

Dickman and Brays reagent

15g ammonium molybdate dissolved in 300ml warm DDW (about 60°C) cooled and filtered. To this 400ml of 10N HCl was added and final volume was made upto 1000ml with DDW.

Diphenyl amine indicator

0.5g diphenyl amine dissolved in a mixture of 20ml DDW and 100ml concentrated H_2SO_4.

EDTA (0.01M)

3.723 disodium salt of ethylene diamine tetra acetic acid dissolved in DDW and diluted to 1000ml.

Erichrome black T indicator

0.4g Erichrome black T ground with 100g powdered sodium chloride.

Ferroin indicator

1.485g 1, 10-phenonthroline monohydrate together with 495 mg $FeSO_4$ dissolved in DDW and volume made upto 100ml.

Ferrous ammonium sulphate (0.5N)

196g hydrated ferrous ammonium sulphate dissolved in DDW. To this 20ml concentrated H_2SO_4 was added and final volume made upto 1000ml.

Folin's phenol reagent

100g sodium tungustate and 25g sodium molybdate dissolved in 700ml DDW to which 50ml 85% phosphoric acid and 100ml concentrated hydrochloric acid was added. The solution was refluxed on heating mantle for 10 hours. At the end, 150g lithium sulphate, 50ml DDW and 3-4 drops liquid bromine added. The reflux condensor was removed and solution was boiled for 15 minutes to remove excess bromine, cooled and diluted upto 1000ml. The strength of this acidic solution was adjusted to 1N by titrating it with 1N sodium hydroxide solution using phenolphthalein as an indicator.

Hydrochloric acid (1N)

86.2ml hydrochloric acid mixed with DDW and final volume was made upto 1000ml.

Isopropanol solution (5%)

5ml isopropanol mixed with 95ml DDW.

Liquid ammonia (1:1)

Ammonia having specific gravity 0.88 diluted with equal amount of DDW.

Manganous sulphate solution

100g manganous sulphate dissolved in boiled DDW and volume made upto 200ml.

Methyl orange indicator

0.5g methyl orange dissolved in 100ml DDW.

Molybdic acid reagent (2.5%)

6.25g ammonium molybdate dissolved in 75ml 10N sulphuric acid. To this solution 175ml DDW was added and the total volume maintained upto 250ml.

Murexide indicator

0.2g ammonium purpurate ground with 100g powdered sodium chloride.

N-(1-Nepthyl) ethylene diamine dihydrochloride (NED-HCl) solution (0.02%)

20mg nepthylethylene diamine dihydrochloride dissolved in sufficient DDW and final volume maintained upto 100ml with DDW.

Nessler's reagent

3.5g potassium iodide dissolved in 100ml DDW to which 4% mercuric chloride solution was added with stirring until a slight red precipitate remained. Thereafter 120g sodium hydroxide with 250ml DDW was added. The volume was made upto 1 litre with DDW. The mixture was filtered twice and kept in an amber coloured bottle.

Olsen's reagent

42.0g sodium bicarbonate dissolved in 1000ml DDW and pH was adjusted to 8.5 with the addition of small quantity of sodium hydroxide.

Phenol disulphonic acid

This was prepared by taking 25g pure phenol (C_6H_5OH, crystal white) in a conical flask (500 ml) to which 150ml concentrated H_2SO_4 and 75ml fuming sulphuric acid were added and kept on boiling water bath for 2 hours. After cooling it was stored in amber coloured bottle.

Phenolphthalein indicator

0.5g phenolphthalein dissolved in 50ml of 95% ethanol to which 50ml DDW was added. Now 0.05N CO_2 free NaOH solution was added dropwise till the solution turned faintly pink.

Phosphate buffer (0.1M) for pH 7.5

(a) 13.6g potassium dihydrogen orthophosphate dissolved in sufficient DDW and final volume made upto 1000 ml with DDW (b) 17.42g dipotassium hydrogen orthophosphate dissolved in sufficient DDW and final volume maintained upto 1000ml with DDW. Now 160ml of solution (a) and 840ml of solution (b) were mixed.

Phosphate buffer (0.2M) for pH 6.8

This was prepared by dissolving 27.80g sodium hydrogen orthophosphate in sufficient DDW and 53.65g disodium hydrogen orthophosphate separately and final volume of each was maintained upto 1000ml with DDW. To get pH 6.8, 5ml of monobasic sodium phosphate solution was mixed with 49ml of dibasic sodium phosphate solution, and diluted to 200ml with DDW.

Potassium chromate indicator (5%)

5g potassium chromate ($K_2Cr_2O_4$) dissolved in DDW, and final volume made upto 100ml.

Potassium dichromate solution (1N)

49.04g potassium dichromate dissolved in 1000ml DDW.

Potassium nitrate solution (0.2N)

2.02g potassium nitrate dissolved in DDW and final volume maintained upto 100ml with DDW.

Reagent A

0.5% copper sulphate solution and 1% sodium tartarate solution mixed in equal volumes.

Reagent B

50 ml 2% sodium carbonate mixed with 1ml reagent A.

Silver nitrate solution

3.4g silver nitrate dissolved in 1000ml DDW.

Sodium hydroxide solution (1N)

4g NaOH dissolved in DDW and final volume made upto 100ml.

Sodium thiosulphate solution (0.025N)

6.2g sodium thiosulphate dissolved in 1000ml DDW.

Solvent mixture

95% ethanol mixed with diethyl ether in 1:1 ratio, and neutralized just before use with KOH (1N) in presence of phenolphthalein solution as indicator

Stannous chloride solution

10g crystalline stannous chloride dissolved in 25ml concentrated HCl by warming and then stored in an amber coloured bottle, giving 40% stannous chloride stock solution, just before use 0.5ml was diluted to 66 ml with DDW.

Starch indicator

1g starch dissolved in 100ml warm (80-90°C) DDW and a few drops of formaldehyde solution were added.

Sulfanilic acid solution

600mg sulfanilic acid dissolved in 70ml warm DDW. After additon of 20ml concentrated HCl, the volume was made upto 100ml.

Sulphanil amide solution

1g sulphanil amide dissolved in 3N 100ml hydrochloric acid.

Sulphuric acid (7N)

190.4ml concentrated sulphuric acid mixed with DDW and final volume made to 1000ml.

Sulphuric acid solution

500ml concentrated H_2SO_4 added to 125ml DDW and cooled.

Microbiological Preparations

1. Nutrient agar (g l^{-1})

Peptic digest of animal tissues	5.00
Beef extract	3.00
Agar	15.00
pH	6.8±0.2

2. EC Broth

Caesin enzymic hydrolysate	20.00
Lactose	5.00
Bile Salt mixture	1.50
Dipotassium phosphate	4.00
Monopotassium phosphate	1.50
Sodium chloride	5.00
pH	6.8±0.2

3. MacConkey Broth

Peptic digest of animal tissue	17.00
Proteose peptone	3.00
Lactose	10.00
Bile salts	1.50
Sodium chloride	5.00
Neutral red	0.003
pH	7.1±0.2

4. Salmonella Shigella (SS) Agar

Peptic digest of animal tissue	5.00
Beef extract	5.00
Lactose	10.00
Bile salt mixture	8.5
Sodium citrate	10.00
Sodium thiosulphate	8.5
Ferric citrate	1.00
Brilliant green	0.0003
Neutral red	0.025
Agar	20.00
pH	7.2

5. Normal Saline Solution

Sodium chloride	0.8g
Water	100 ml
pH	6.8±0.2